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TOXICITY OF NITRO-HETEROCYCLIC AND NITROAROMATIC ENERGETIC MATERIALS TO TERRESTRIAL PLANTS IN A NATURAL SANDY LOAM SOIL

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14. ABSTRACT
The United States Environmental Protection Agency is developing ecological soil screening level (Eco-SSL) values for ecological risk assessment of contaminants at Superfund sites. Insufficient information for RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB to generate Eco-SSLs for terrestrial plants necessitated toxicity testing to fill the data gaps. Standardized toxicity tests were selected and used, on the basis of their ability to measure chemical toxicity to ecologically relevant test species, and their inclusion of growth component among the measurement endpoints. Tests were conducted in Sassafras sandy loam soil, which supports relatively high bioavailability of the energetic materials. Weathering/aging of amended treatment soil was incorporated in the study to better reflect the exposure conditions in the field soils. Definitive toxicity tests conducted with freshly amended and weathered/aged amended soils generated ecotoxicological benchmarks, including EC_{20} values for growth that can be used for Eco-SSL development. These study results will be provided to the Eco-SSL workgroup for review and for developing ecological soil screening levels (Eco-SSLs) for RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB.

15. SUBJECT TERMS

RDX	TNB	Ecological soil screening level	Bioavailibilty
HMX	Toxicity assessment	Terrestrial plants	
2,4-DNT	Weathering/aging	Natual soil	

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PREFACE

The work described in this report was authorized under Project No. SERDP CU-1221. The work started in April 2001 and was completed in August 2003.

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TOXICITY OF NITRO-HETEROCYCLIC AND NITROAROMATIC ENERGETIC MATERIALS TO TERRESTRIAL PLANTS IN A NATURAL SANDY LOAM SOIL

1. INTRODUCTION

The Strategic Environmental Research and Development Program (SERDP) identified a research need under the FY00 Broad Agency Announcement (BAA) CUSON-SP-00-04, "Development of Ecological Toxicity and Biomagnification Data for Explosives Contaminants in Soil," to extend the knowledge of the toxicity of explosives-related soil contaminants to ecological receptors. Ecological receptors of interest included terrestrial plants and soil invertebrates. The focus of this investigation was to obtain direct experimental data on toxicity of nitro-heterocyclic and nitroaromatic compounds to terrestrial plants in soil with parameters (i.e., pH, organic matter, clay content, etc.) promoting a relatively high bioavailability of the energetic materials (EM).

Many scientists have investigated the toxicity of 2,4,6-trinitrotoluene (TNT) to plants, but few have investigated the phytotoxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 1,3,5-trinitrobenzene (TNB) or dinitrotoluenes. Phytotoxicity of TNT has been evaluated using single aquatic species, (i.e., Eurasian Watermilfoil and duckweed), and terrestrial plant species (i.e., yellow nutsedge, poplar, lettuce, and tall fescue (Schott and Worthley, 1974; Palazzo and Leggett, 1986; Cataldo *et al.*, 1989; Peterson *et al.*, 1996; Pavlostathis *et al.*, 1998; Thompson *et al.*, 1998; Sunahara *et al.*, 2001). Toussaint *et al.* (1995) reported an EC_{50} value of 1 μM (0.2 mg L^{-1}) for the effect of TNT on lettuce root elongation. Robidoux *et al.* (2003) estimated IC_{20} values of 204 and 3113 mg kg^{-1} TNT for lettuce seedling emergence in forest soil and artificial soil (silica), respectively. Exposure of barley seeds to TNT in forest soil produced IC_{20} values of 398, 139, 272, and < 91 mg kg^{-1} TNT for barley seedling emergence, fresh shoot mass, dry shoot mass, and root mass, whereas these values were 8133, 8133, 133, 1199, and < 56 mg kg^{-1} TNT in artificial soil (silica) (Robidoux *et al.*, 2003).

Other studies compared the toxicity of different plant species to TNT, RDX, and TNB, individually. Gong *et al.* (1999) compared the toxicity of TNT to cress, turnip, oat, and wheat and determined a lowest observable adverse effect concentration (LOAEC) of 50 mg kg^{-1} TNT in soil and stimulation of seedling growth at lower concentrations of TNT (5 to 50 mg kg^{-1}). Scheidemann *et al.* (1998) showed that alfalfa could not grow in soil contaminated with 100 mg kg^{-1} TNT, whereas wheat and bush bean could develop at 500 mg kg^{-1} TNT in soil. Winfield *et al.* (1999) found that sunflower and sanfroin were the most sensitive species among ten species exposed to RDX at soil concentrations up to 4000 mg kg^{-1}. Reddy *et al.* (1994) assessed the toxicity of TNB in sand using lettuce and oat. The authors reported seed germination EC_{50} values of 19 mg kg^{-1} for lettuce and > 375 mg kg^{-1} for oat.

Few studies investigated the toxicity of EM mixtures to terrestrial plants. In a study of collected field soils, Simini *et al.* (1995) compared the toxicity of soils contaminated with TNT, TNB, RDX, HMX, and heavy metals to cucumber and radish. They determined that toxicity was mostly related to TNT and TNB, with a LOEC of 7 to 19 mg kg^{-1} TNT in soil. In another field study (Price *et al.*, 1997; Pennington and Brannon, 2002), corn stover was more tolerant compared with tomato vine, nutsedge, corn ears, tomato fruit, and lettuce. In that study, corn, tomato, and lettuce died when exposed to 580 mg kg^{-1} RDX and 1720 mg kg^{-1} TNT. All these studies demonstrated that phytotoxicity of explosives was species dependent, but no generalization for sensitivity between monocotyledonous and dicotyledonous plants could be drawn.

Soil type also influences the chemical bioavailability and toxicity of a contaminant. In a comparative study of either TNT or HMX toxicities to lettuce and barley, using artificial and forest soils, Robidoux *et al.* (2003) determined that TNT was more toxic to barley in organic forest soil than in mineral artificial (silica) soil. The HMX was not toxic to lettuce and barley up to 1866 mg kg^{-1} HMX in artificial soil and up to 3320 mg kg^{-1} HMX in forest soil. A 45-day exposure to 80 mg kg^{-1} TNT in Tunica silt or Sharkey clay soils, did not affect yellow nutsedge growth compared with controls (Pennington, 1988; Talmage *et al.*, 1999).

Review of the literature showed that, except for TNT, few studies have sufficiently investigated the effects of EMs on terrestrial plants, although these contaminants are persistent and some are highly mobile in the environment. As a result, no screening values, which could be used in the ecological risk assessment (ERA), are available for these EM soil contaminants. Scientifically based ecological soil screening levels (Eco-SSLs) are needed to identify EM concentrations in soil that present an acceptable ecological risk. The Eco-SSLs are defined as concentrations of chemicals in soil that, when not exceeded, will be protective of terrestrial ecosystems from unacceptable harmful effects. These Eco-SSL concentrations can be used in a screening level ERA to identify those contaminants in soil that warrant additional evaluation in a baseline ERA and eliminate those that do not. The insufficient information for EMs required to generate Eco-SSLs for terrestrial plants necessitated this study to fill this knowledge gap.

This study was designed to produce benchmark data for the development of Eco-SSLs for RDX, HMX, 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), and TNB for terrestrial plants and meet specific criteria [(United States Environmental Protection Agency (USEPA), 2000)], including:

- tests conducted in soil having physico-chemical characteristics that support relatively high bioavailability of chemicals
- experimental designs for laboratory studies were documented
- nominal and analytically determined concentrations of chemicals of interest were reported
- tests included negative and positive controls
- tests that included growth measurement endpoint were used
- appropriate chemical dosing procedures were reported

- concentration-response relationships were reported
- statistical tests used to calculate the benchmark and level of significance were described
- the origin of test species were specified.

The specific objectives of this study included the assessing EM toxicity by determining the bounded no oberserved effect concentration (NOEC) and lowest observable effect concentration (LOEC) values, and EC_{20} and EC_{50} values for plant germination and growth measurement endpoints based on concentration-response relationships; evaluating soil extraction methods to determine which chemical measure of exposure better correlates with toxicity; and examining the potential effects of weathering and aging of amended soil on EM toxicity to terrestrial plants.

2. MATERIAL AND METHODS

2.1 Sassafras Sandy Loam Soil.

A natural soil, Sassafras sandy loam [fine-loamy, siliceous, mesic Typic Hapludult] (SSL) was used in this study to assess the toxicity of test chemicals to plants. This soil was selected for developing ecotoxicological values protective of soil biota because it has physical and chemical characteristics supporting relatively high bioavailability of the test chemicals (low organic matter and clay contents). The SSL soil was collected from an open grassland field on the property of the U.S. Army Aberdeen Proving Ground (APG), Edgewood Area. Vegetation and the organic horizon were removed to just below the root zone, and the top 6-in. of the A horizon was then collected. The soil was sieved through a 5 mm^2 mesh screen, air-dried for at least 72 hr mixed periodically to ensure uniform drying, and then stored at room temperature before testing. The soil was analyzed for physical and chemical characteristics by the Cooperative Extension Service, University of Maryland Soil Testing Laboratory (College Park, MD). Results of these analyses are presented in Table 1.

Table 1. Physical and Chemical Characteristics of Sassafras Sandy Loam Soil Analyzed by the Cooperative Extension Service, University of Maryland Soil Testing Laboratory, College Park, MD

Soil Parameter	Sassafras Sandy Loam
Sand (%)	69
Silt (%)	13
Clay (%)	17
Texture	Sandy loam
CEC (cmol $kg^{-1)}$	5.5
Organic matter (%)	1.2
pH	5.2

2.2 Chemicals.

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX; CAS: 121-82-4; Purity: 99%), HMX (CAS: 2691-41-0; Purity: 99%), and TNB (CAS: 99-35-4; Purity: 99.7%) were obtained from the Defense Research Establishment Valcartier of the Canadian Ministry of National Defense (Val Bélair, Quebec, Canada). The 2,4-dinitrotoluene (2,4-DNT; CAS: 121-14-2; Purity: 97%), and 2,6-DNT (CAS: 606-20-2; Purity: 98%) were obtained from Sigma-Aldrich Canada (Oakville, Ontario, Canada). Boric acid (H_3BO_3; CAS: 10043-35-3; Purity: 99.9%) was used as the positive control. Acetone (CAS: 67-64-1; HPLC Grade) was used for preparing EM solutions during soil amendments. Acetonitrile (CAS: 75-05-8; HPLC Grade), calcium chloride ($CaCl_2 \cdot 2H_2O$; analytical grade), and sodium bisulfate ($NaHSO_4 \cdot H_2O$; certified grade) were used for extractions for chemical analyses, and 1,3-dinitrobenzene (1,3-DNB) was used as the internal standard. Glassware was washed with phosphate-free detergent, followed by rinses with acetone, nitric acid, and ASTM Type I water (American Society of Testing and Materials, http://www.astm.org). The ASTM Type I water was obtained using Millipore® Super Q water purification system (Millipore®, Nepean, Ontario, Canada) and was used throughout the study.

2.3 Soil Amendment Procedure.

The SSL soil was individually amended with either RDX, HMX, 2,4-DNT, 2,6-DNT, or TNB. Prepared SSL soil was weighed separately for each treatment in a glass dish. For each treatment, soil was spread to a thickness of approximately 2.5 - 4 cm. Each concentration of EM was prepared separately in glass volumetric flasks and dissolved in acetone. The EM/acetone solution was quantitatively transferred to the soil evenly across the soil surface, ensuring that the volume of solution added at any one time did not exceed 15% (volume $mass^{-1}$) of the dry mass soil. After addition of the EM solution, the volumetric flask was rinsed twice with a known volume of acetone and this was also applied to the soil surface. The solution was added in successive stages, each time allowing the acetone to evaporate for a minimum of 2 hr, if the total volume of solution needed to attain the target EM concentration in soil exceeded 15% (v/m^{-1}). The same total EM/acetone solution volume was added to every treatment, equaling the volume required to dissolve the EM at the highest concentration tested. The amended soil was then air-dried overnight (minimum of 18 hr) in a darkened chemical hood. Each amended soil sample was transferred into a high-density polyethylene container coated with fluoropolymer (Teflon®-like chemical) and covered with aluminum foil to prevent photolysis of the EM. The sample was mixed overnight (18 ± 2 hr) using a three-dimensional mixer. Soil was then ready for the phytotoxicity assays.

Weathered/aged amended soil was prepared in the same manner as the freshly amended soil. The ASTM Type I water was added to adjust the soil moisture to a level equivalent to 75 % of the water holding capacity (WHC). Hydrated soil was exposed to wetting and drying cycles and sunlight in a greenhouse for a period of 13 weeks. Each week, ASTM Type I water was added to adjust the soil moisture to initial level (75% of WHC). The hydrated soil was allowed to dry until the next addition of water. The week before the initiation of plant toxicity test using weathered/aged amended soil, each air-dried soil treatment was mixed overnight using a three-dimensional mixer 1 day prior to the initiation of the test.

2.4 Water Holding Capacity of Soil.

Water holding capacity of the soil was measured accordingly to the procedure provided by Dr. Ronald Checkai (ECBC). The SSL soil was transferred into 10 cm plastic pots in triplicate so that the soil surface was 2 cm below the rim of the pot. Pots were placed on 2 mm mesh sieves to allow free water drainage. A volume of ASTM Type I water equal to the soil volume was slowly added onto the settled soil. Water was allowed to dry for 24 hr. A first aliquot of soil was sampled 1-3 cm below the soil surface. Moist soil was immediately weighed and recorded as wet mass (Mass $_{moist\ soil}$). Similar aliquots were taken from the two other replicates. Sub-samples of the moist soil were dried in a 105 °C oven for 18 hr. The samples were then transferred in a desiccator at room temperature for 30 min prior to weighing the dry mass (Mass $_{dry\ soil}$). This procedure was repeated after 48 hr and 72 hr ensuring a steady state for WHC had been achieved. The WHC was calculated according to the following formula:

$$WHC\ \% = [(Mass_{moist\ soil} - Mass_{dry\ soil}) / Mass_{dry\ soil})] * 100$$

2.5 Measurement of Soil pH.

Using freshly amended soil, the soil pH was measured in each treatment concentration at the beginning of each range-finding tests. Using freshly amended and weathered/aged amended soils, the soil pH was also measured in each treatment concentration at the beginning and end of each definitive tests. The soil pH was measured according to ISO 10390 method (International Standardization Organization, 1994). Approximately 5 mL vol of soil was placed in a 50-mL tube and 25 mL of ASTM Type I water was added. The sample was vortexed for 20 sec and rotated for 5 min at 90 rpm. Soil slurry was let stand at 21 ± 3 °C for 3 hr prior to measurement. The pH reading was taken after 1 min, which was sufficient to have a stable reading.

2.6 Measurement of Soil Redox Potential.

The oxidation-reduction (redox) potential was measured in each treatment concentration at the beginning (one reading per concentration) and at the end of each definitive test, using freshly amended and weathered/aged amended soils (three replicate per concentration and per plant species). The redox potential of soil was measured according to the supplier's instruction (Accumet; Patrick *et al.*, 1996). Prior to redox measurement, soil samples were equilibrated with ASTM Type I water (75% of the WHC) during 24 hr, in the dark at room temperature. Redox readings were taken after 5 min, which was sufficient to obtain a stable reading.

2.7 Cation Exchange Capacity of Soil.

At the beginning of each definitive test, using freshly amended and weathered/aged amended soils, soil cation exchange capacity was measured in duplicate for each treatment concentration. The effective cation exchange capacity (CEC) was measured according to Hendershot *et al.* (1993) and was performed by Hélène Lalande at McGill University (Montreal, Quebec, Canada). An aliquot of 0.5 - 3.0 g of air-dried soil (< 2 mm) was weighed in

a 50-mL centrifuge tube, in duplicate. To each tube, 30.0 mL of 0.1 M of $BaCl_2$ was added and shaken slowly on an end-over-end shaker (15 rpm) for 2 hr. Each tube was centrifuged (15 min, 700 x g), and the supernatant was filtered with Whatman No. 41 filter paper. The cations Ca, Mg, K, Na, Al, Fe, and Mn were analyzed with an atomic absorption spectrophotometer. Effective CEC was calculated using the following equation:

$$\text{Effective CEC cmol (+) kg}^{-1} = \Sigma\, M^{+} \text{ cmol (+) kg}^{-1}$$

2.8 Soil Acetonitrile Extraction.

Acetonitrile extractions of soil samples were performed at the beginning of each range-finding test using freshly amended soil. At the beginning and end of each definitive test acentonitrile extraction of soil samples were perfromed using freshly amended and weathered/aged amended soils. Acetonitrile extraction procedure is a modification of the Method # 8330A (USEPA, 1998). At the beginning of each toxicity test and after addition of ASTM Type I water (75% of WHC), soil samples were equilibrated in the dark for 24 hr at room temperature. Aliquots of 2.0 g were sampled in triplicate from each treatment concentration. At the end of each definitive toxicity test, aliquots of 2.0 g were taken from each treatment replicate. One hundred microliters of 50 mg 1,3-dinitrobenzene (1,3-DNB) L^{-1} internal standard solution and 10 mL of acetonitrile were added to each soil aliquot placed in individual glass tubes. Glass tubes were vortexed for 1 min and then sonicated in the dark for 18 hr ± 2 hr at 20 °C. Five milliliters of sonicated sample was transferred to a new tube and 5 mL of 5 g L^{-1} $CaCl_2$ solution was added. For soil samples amended with TNB, a solution of 5 g L^{-1} $CaCl_2$ + 0.2 g L^{-1} $NaHSO_4$ was added to prevent TNB degradation. Supernatant was filtered through 0.45 µm Millex-HV cartridges. Soil extracts were analyzed and quantified using an high performance liquid chromatography (HPLC). Extraction was repeated if 1,3-DNB internal standard recovery was lower than 90%.

2.9 Soil ATCLP Extraction.

In addition to acetonitrile extraction, soil samples were extracted using an Adapted Toxicity Characteristic Leaching Procedure (ATCLP) (Haley *et al.*, 1993) at the beginning of each definitive test, using freshly amended and weathered/aged amended soils. The ATCLP is based on modification of the Toxicity Characteristic Leaching Procedure (TCLP) (40 CFR Part 268.41, Hazardous Waste Management, Method 1311). The modification involved substitution of CO_2-saturated ASTM Type I water for acetic acid which was better simulating field soil-water condition due to respiration by soil biota. Prior to ATCLP extraction and after addition of ASTM Type I water (75% of WHC), soil samples were equilibrated in the dark for 24 hr at room temperature. For each treatment concentration, aliquots of 4.0 g soil were transferred in triplicate into 20-mL scintillation vials. Sixteen milliliters of CO_2 saturated water at pH 4.5 was added, and vials were rapidly sealed tight. Soil samples were vortexed 45 sec and were mixed in the dark for 18 ± 2 hr using a rotary mixer (30 rpm) at room temperature. Soil was allowed to settle and supernatants were filtered through .45 µm Millex-HV cartridges. An equivalent volume of acetonitrile was added to filtered soil extract prior to HPLC analysis.

For TNB soil extracts, an equivalent volume of acetonitrile (0.2 g L^{-1} $NaHSO_4$ solution 1:1) was added. In this report, ATCLP soil extraction is referred to as the water-soluble fraction of EM, which was perceived to measure the portion of EM bioavailable to plants.

2.10 Chemical Analysis.

Soil and plant extracts were analyzed using a Thermo Separation Products chromatographic system composed of model P4000 pump, a model AS1000 injector (including the temperature control for the column), and a model UV6000LP photodiode-array detector. For TNB, 2,4-DNT and 2,6-DNT analyses, a Supelcosil C8 column (25 cm x 4.6 mm ID, 5 μm particles) and an 18% 2-propanol / 82% water mobile phase were used. The flow rate was 1 mL min^{-1} and the run time was 40 min. For RDX and HMX analyses, the column used was a Supelcosil LC-CN (25 cm x 4.6 mm ID, 5 μm), held at 35 °C. The initial solvent composition was 30% methanol / 70% water, which was held for 8 min. A linear gradient was then run from 30 to 65% methanol over 12 min. This solvent ratio was then changed to initial conditions (30% methanol) over 5 min. These initial conditions were held for an additional 5 min. The injection volume was 50 μL. The detector was set to scan from 200 to 350 nm and chromatograms were extracted at 254 nm. The limit of quantification was 50 ppb for each chemical.

2.11 Plant Toxicity Tests.

The plant toxicity tests were performed according to protocols of ASTM Standard Guide for conducting terrestrial plant toxicity tests (American Society for Testing and Materials, 1998) and USEPA early seedling growth test (USEPA, 1982).

Range-finding tests were performed using Kandy corn Canada No. 1, *Zea mays* (Williams Dam Seeds Ltd., Dundas, Ontario, Canada); lettuce variety Buttercrunch, *Lactuva sativa* (Stokes Seeds Ltd, Thorold, Ontario, Canada); alfalfa variety Canada No. 1, *Medicago sativa* (Williams Dam Seeds Ltd., Dundas, Ontario, Canada); perennial ryegrass variety Express, *Lolium perenne* (Pickseed Canada Inc., St. Hyacinthe, Quebec, Canada); and Japanese millet variety Common No. 1, *Echinochloa crusgalli* (Labon Inc. Boucherville, Quebec, Canada). Five nominal concentrations 1, 10, 100, 1000, and 10000 mg kg^{-1}, as well as negative control (ASTM Type I water) and a carrier control (acetone), were tested in triplicate. The soil was amended as described in Section 2.3. Twenty seeds of each plant species were sown in a 10-cm pot containing 200 g dry soil except for corn. Seven seeds of corn were sown in a 10-cm pot containing 200 g dry soil. The bottom of each plant pot was covered with a piece of cheesecloth to prevent soil loss during testing. Alfalfa seeds were inoculated with nitrogen-fixing bacteria prior to sowing. Thirty milliters of ASTM Type I water was added to obtain 75% of WHC. Plant pots were placed in 1-L polyethylene bags and closed with an elastic band to minimize loss of soil water due to evapo-transpiration. Plant toxicity tests were performed in a temperature and light controlled growth chamber. Plants were incubated in the dark for the first 2 days and then exposed to a normal diurnal cycle afterwards. The growth chamber conditions were set as follows: light intensity at 5000 ± 500 lux, day time at 25 °C for 16 hr, and night time at 20 °C for 8 hr. Luminosity level was measured weekly using a photometer. The light intensity was adjusted when needed.

Based on the results of range-finding tests, definitive tests were performed using the three most sensitive plant species, with four replicates per treatment. The most sensitive plant species tested were alfalfa (*Medicago sativa*), perennial ryegrass (*Lolium perenne*), and Japanese millet (*Echinochloa crusgalli*). Six to nine nominal concentrations, as well as negative control (ASTM Type I water) and a carrier control (acetone), were used.

The numbers of emerged seedlings for alfalfa, Japanese millet and corn, were counted after 5 days and for lettuce and ryegrass, after 7 days. Shoot number, shoot fresh mass, and shoot dry mass for alfalfa, Japanese millet, and corn were measured after 16 days. Shoot number, shoot fresh mass, and shoot dry mass for lettuce and ryegrass were measured after 19 days. Shoot dry mass was obtained after drying at 70 °C for 24 ± 2 hr. Reference toxicant, boric acid, was used as the positive control (ASTM, 1998). Definitive toxicity tests were repeated when the percentage of germination in the controls were lower than 85% for ryegrass or Japanese millet, or lower than 70% for alfalfa, and when boric acid EC_{50} values were outside the quality control limit equivalent to EC_{50} average value ± 2 times standard deviation.

2.12 Statistical Methods.

The EC_{20} and EC_{50} values for seedling emergence, shoot fresh mass, and shoot dry mass measurement endpoints were calculated using SYSTAT software, version 7.0 (SPSS Inc., 1997). Histograms of the residuals and stem-and-leaf graphs were examined to ensure that normality assumptions were met. Variances of the residuals were examined to decide whether or not to weight the data and to select potential models. The following nonlinear regression models were used:

Logistic Gompertz model: $Y = a \times e^{([\log(1-p)] \times [C/ECp]^b)}$

Exponential model: $Y = a \times e^{(([\log(1-p)] / ECp) \times C)} + b$

Logistic Hormetic model: $Y = (t \times [1 + hC] / \{1 + [(p + h\,ECp) / (1 - p)] \times [C/ECp]^b\}$

where Y is the number of emerged seedlings or the shoot mass; a is the control response; t is the control response in the hormetic model; e is the base of the natural logarithm; p is the percent inhibition/100 (e.g., 0.5 for EC_{50}); C is the exposure concentration in test soil; ECp is the estimate of effect concentration for a specified percent effect; h is the hormetic effect parameter; and b is the scale parameter. The ECp parameters used in this study included the EM concentration producing either a 20% (EC_{20}) or 50% (EC_{50}) reduction in the measurement endpoint. The EC_{20} parameter, based on a growth endpoint, is the preferred parameter for deriving terrestrial plant Eco-SSL benchmarks. The EC_{50}, a commonly reported value, was included to enable comparisons of the results produced in this study with results reported by other researchers. The asymptotic standard error (a.s.e.) and 95% confidence intervals (CI) associated with the point estimates were determined. The raw R-squared values, which reflect the variation of the measurement endpoints (dependent variable) that is explained by the chemical concentration (independent variable), were reported.

Analysis of Variance (ANOVA) was used to determine the bounded NOEC and LOEC values for germination or growth data. Mean separations were done using Fisher's Least Significant Difference (LSD) pairwise comparison tests. When no observed adverse effect concentration (NOAEC) or (lowest observed adverse effect concentration) (LOAEC) values were determined, which usually happened in tests with hormetic response at low exposure concentrations of chemicals, the same statistical methods were used. A significance level of $p < 0.05$ was accepted for determining the NOEC and LOEC values. Student's *t*-Test (two-tailed) with significance level set at $p < 0.05$ was used in the limit tests with plants exposed to RDX or HMX using EXCEL software (Microsoft Corporation, 1997). All analyses were conducted using measured EM concentrations.

3. RESULTS

3.1 EM Concentrations in Range-Finding Toxicity Tests.

Analytical determinations of EM soil concentrations, using acetonitrile extractions of freshly amended soils in the range-finding tests, showed relatively good concordance between nominal and measured concentrations (Table 2). Measured/nominal ratio ranged from 0.80 to 1.17. Higher discrepancy determined for the 10 mg kg^{-1} RDX amended soil may be due to the presence of residual ethanol, which is the solvent in which RDX was stored. Variation within each concentration was lower then 10%, indicating a relatively low variation among the three replicates.

3.2 Physico-Chemical Characterization of Sassafras Sandy Loam Soil.

Soil pH, redox potential, and CEC were measured at the beginning of each definitive test. Results are presented in Tables 3 through 10. Initial soil pH values ranged from 5.8 to 6.2 in the negative controls, 5.9 to 6.2 in the carrier controls, 5.5 to 6.2 in the soil freshly amended with the five EMs, and 5.7 to 6.3 in the weathered/aged amended soil. No significant difference was observed among controls and soil exposed to the different Ems. There was no correlation observed between the pH values and concentrations of EMs.

Initial redox potentials ranged from 281 to 463 in the negative controls, 295 to 473 in the carrier controls, 316 to 481 in soil freshly amended with energetic compounds, and 241 to 347 the weathered/aged amended soil exposed to energetic compounds. Although the redox variation within each definitive test was broad, no significant difference was observed among controls and soil amended with different energetic compounds. There was no correlation observed between redox values and concentrations of energetic compounds.

Text continues on page 26.

Table 2. Acetonitrile Soil Extraction of Range-Finding Tests (n = 3)

Chemical	Nominal Value (mg kg^{-1} soil)	Measured Value (mg kg^{-1} soil)	Standard Deviation	Deviation (%)	Measured/Nominal Ratio
HMX	10	10.5	0.4	4.3	1.05
	100	97.7	1.4	1.4	0.98
	1000	1025	58	5.7	1.02
	10000	9930	830	8.3	0.99
RDX	10	15.3	9.5	61.8	1.53
	100	90.9	1.4	1.6	0.91
	1000	800	38	4.7	0.80
	10000	8550	155	1.8	0.85
TNB	10	11.7	0.3	2.9	1.17
	100	115.8	5.5	4.9	1.16
	1000	1083	33	3.0	1.08
	10000	10620	315	2.9	1.06
2,4-DNT	10	10.2	0.6	5.6	1.02
	100	94.7	4.4	4.7	0.95
	1000	967	99	10.2	0.97
	5000	4900	320	6.6	0.98
2,6-DNT	10	9.6	0.6	6.3	0.96
	100	100.0	5.4	5.4	1.00
	1000	970	38	3.9	0.97
	5000	4900	530	10.9	0.98

Table 3. Initial Soil pH, Redox Potential, and CEC in SSL Soil Used for Freshly Amended TNB Definitive Plant Toxicity Test

TNB Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	5.88	463.4	3.161
Control (carrier)	5.93	473.3	3.139
Average of controls	**5.91**	**468.4**	**3.150**
2	5.83	445.8	2.817
5	6.04	466.8	3.190
10	5.72	471.9	3.132
20	5.77	461.8	3.035
40	5.97	462.9	2.859
60	6.01	464.9	2.849
80	6.06	469.6	3.123
120	6.04	476.8	2.941
160	6.11	471.8	3.035
250	5.75	480.7	2.974
320	6.2	472.8	2.861
600	5.93	482.2	2.897
800	5.52	478.9	3.189
Average of TNB soil	**5.92 ± 0.05***	**469.8 ± 2.7***	**2.992 ± 0.037***

*values are mean ± standard error.

Table 4. Initial Soil pH, Redox Potential, and CEC in SSL Soil Used for Weathered/Aged TNB Definitive Plant Toxicity Test

TNB Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	5.96	332.8	3.23
Control (carrier)	5.95	352.5	3.13
Average of controls	**5.96**	**342.7**	**3.18**
2	5.93	338.0	3.20
5	5.93	323.5	3.22
10	6.00	347.3	3.26
20	6.00	329.4	3.05
40	5.93	305.4	3.11
60	5.95	292.6	3.12
80	5.95	296.2	3.25
120	5.82	317.5	3.27
160	5.77	305.5	3.20
250	5.84	312.6	3.22
320	5.86	309.9	3.25
600	5.79	310.0	3.13
800	5.77	303.1	2.98
1200	5.91	314.9	3.18
1600	5.86	318.1	3.19
Average of TNB soil	**5.89 ± 0.02***	**314.9 ± 4.0***	**3.18 ± 0.02***

*values are mean ± standard error.

Table 5. Initial Soil pH, Redox Potential, and CEC in SSL Soil Used for Freshly Amended 2,4-DNT Definitive Plant Toxicity Test

2,4-DNT Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	5.94	322.5	2.85
Control (carrier)	5.87	318.2	3.12
Average of controls	**5.91**	**320.4**	**2.99**
0.5 - ryegrass repeat	5.84	387.3	
1	5.81	362.8	3.47
1 - ryegrass repeat	5.87	391.0	
2	5.90	316.0	3.55
2.5 - ryegrass repeat	5.87	387.1	
5	5.85	330.8	3.64
5 - ryegrass repeat	5.87	385.5	
10	5.83	335.0	3.44
10 - ryegrass repeat	5.95	368.1	
25	5.88	348.2	3.43
40 - ryegrass repeat	5.98	378.6	
50	5.88	361.7	3.53
100	5.88	373.3	3.53
300	5.87	369.2	3.27
600	5.90	361.2	3.18
Average of 2,4-DNT soil	**5.88 ± 0.01***	**363.7 ± 5.8***	**3.45 ± 0.05***

*values are mean ± standard error.

Table 6. Initial Soil pH, Redox Potential, and CEC in SSL Soil Used for Weathered/Aged 2,4-DNT Definitive Plant Toxicity Test

2,4-DNT Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	5.82	308.0	2.958
Control (carrier)	6.00	332.7	3.056
Average of controls	**5.91**	**320.4**	**3.007**
5	6.11	341.9	2.842
10	6.09	344.6	2.898
25	6.15	312.2	2.984
50	6.11	287.2	2.783
100	6.15	298.9	2.593
200	6.25	293.8	2.772
300	6.22	303.1	3.000
600	6.36	304.9	3.290
1200	6.33	313.4	3.370
Average of 2,4-DNT soil	**6.20 ± 0.03***	**311.1 ± 6.7***	**2.948 ± 0.083***

*values are mean ± standard error.

Table 7. Initial Soil pH, Redox Potential, and CEC in SSL Soil Used for Freshly Amended 2,6-DNT Definitive Plant Toxicity Test

2,6-DNT Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	6.17	436.6	3.540
Control (carrier)	6.19	370.3	3.378
Average of controls	**6.18**	**403.5**	**3.459**
1	6.12	425.7	3.777
2	6.17	425.3	3.898
5	6.16	403.3	3.719
10	6.1	404.1	3.622
20	6.08	418.5	3.755
40	6.08	375.2	3.619
100	6.12	405.5	3.602
500	5.98	392.9	3.638
Average of 2,6-DNT soil	**6.10 ± 0.02***	**406.3 ± 6.0***	**3.704 ± 0.036***

*values are mean ± standard error.

Table 8. Initial Soil pH, Redox Potential, and CEC in SSL Soil Used for Weathered/Aged 2,6-DNT Definitive Plant Toxicity Test

2,6-DNT Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	5.92	344.7	2.958
Control (carrier)	5.99	294.7	3.122
Average of controls	**5.96**	**319.7**	**3.040**
2	6.08	265.8	3.131
5	6.06	302.7	3.037
10	6.11	306.3	2.901
20	6.06	310.1	3.001
40	6.18	287	3.063
50 - ryegrass repeat	5.72	244.4	
100	6.09	314.5	2.938
100 - ryegrass repeat	5.68	240.7	
150 - ryegrass repeat	5.68	263.2	
200	6.02	280.5	3.274
200 - ryegrass repeat	5.69	280	
250 - ryegrass repeat	5.71	283.9	
300 - ryegrass repeat	5.76	268.5	
500	5.99	304.8	3.109
1000	5.93	298.3	3.102
Average of 2,6-DNT soil	**5.92 ± 0.05***	**283.4 ± 6.0***	**3.062 ± 0.037***

*values are mean ± standard error.

Table 9. Initial Soil pH, Redox Potential, and CEC in Definitive Plant Toxicity Test in SSL Soil Used for RDX or HMX Freshly Amended Definitive Toxicity Tests

Nominal Concentration (mg kg^{-1})	pH	Redox Potential (mV)	Cation-Exchange Capacity (cmol kg^{-1})
Control (negative)	6.19	318	3.031
Control (carrier)	5.98	313	3.086
Average of controls	**6.09**	**315.5**	**3.059**
RDX 10000	6.01	324	3.202
HMX 10000	6.05	336	3.178

Table 10. Initial Soil pH, Redox Potential, and CEC in Definitive Plant Toxicity Test in SSL Soil Used for RDX or HMX Weathered/Aged Definitive Toxicity Tests

Nominal Concentration ($mg\ kg^{-1}$)	pH	Redox Potential (mV)	Cation-Exchange Capacity ($cmol\ kg^{-1}$)
Control (negative)	5.91	281.3	2.719
Control (carrier)	5.96	308.2	2.879
Average of controls	**5.94**	**294.8**	**2.799**
RDX 10000	5.95	312.9	2.941
HMX 10000	5.96	307.4	3.032

Initial CEC values ranged from 2.9 to 3.5 in the negative controls, 3.1 to 3.4 in the carrier controls, 2.8 to 3.9 in the freshly amended soil, and 2.6 to 3.4 in the weathered/aged amended soil. No significant difference was observed among controls, and soil amended with the different energetic compounds. There was no correlation observed between CEC values and concentrations of EMs.

At the end of test with TNB freshly amended soil, the soil pH was measured in triplicate for each concentration and each plant species (Table 11). Because pH variation was low among replicates, soil pH was measured in one sample per concentration and per species in the remaining definitive tests. Redox potential was measured using three replicates per concentration and per plant species. The results are presented in Tables 12 through 18.

Significant differences ($p < 0.05$) between pH values measured at the beginning and end of phytotoxicity tests were observed in most soil exposed to the different EMs, except for ryegrass (Table 19). For ryegrass, the pH difference was significant only in either 2,4-DNT or 2,6-DNT freshly amended soils.

Significant differences ($p < 0.05$) between redox values measured at the beginning and end of phytotoxicity tests were also observed in most amended soils (Table 19). However, there was no significant difference in redox values in TNB freshly amended soil for all three plant species, no difference in weathered/aged TNB amended soil, as well as in 2,4-DNT freshly amended soil for Japanese millet and ryegrass, respectively; and in weathered/aged 2,4-DNT amended soil for alfalfa and Japanese millet.

Text continues on page 35.

Table 11. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Freshly Amended TNB Definitive Plant Toxicity Test

TNB Concentration (mg kg^{-1})	pH - Alfalfa	pH - Japanese Millet	pH - Ryegrass	Redox Potential – Alfalfa	Redox Potential - Japanese millet
Control (negative)	5.46 ± 0.08	5.82 ± 0.08	5.86 ± 0.03	435.1 ± 0.9	489.2 ± 3.6
Control (carrier)	5.48 ± 0.05	5.87 ± 0.05	5.83 ± 0.05	467.3 ± 0.8	463.4 ± 14.9
Average of controls	**5.47 ± 0.01***	**5.84 ± 0.02***	**5.84 ± 0.01***	**451.2 ± 16.1***	**476.3 ± 12.9***
2		5.93 ± 0.03	5.87 ± 0.02		479.9 ± 4.1
5	5.66 ± 0.02	5.82 ± 0.02		465.1 ± 0.7	479.9 ± 4.7
10		5.90 ± 0.02	5.96 ± 0.03		467.9 ± 4.0
20		5.86 ± 0.04	5.95 ± 0.02		472.4 ± 0.1
40	5.74 ± 0.04		6.05 ± 0.02	462.7 ± 3.9	
60		5.89 ± 0.02			460.8 ± 0.9
80	5.85 ± 0.04			457.2 ± 1.1	
120		5.72 ± 0.02	5.72 ± 0.04		450.3 ± 6.6
160	5.85 ± 0.02			461.0 ± 5.1	
250		5.57 ± 0.01	5.69 ± 0.04		470.9 ± 1.4
320	5.51 ± 0.06			467.8 ± 1.3	
600	5.64 ± 0.03	5.59 ± 0.04	5.60 ± 0.01	473.8 ± 0.9	468.7 ± 0.4
800	5.64 ± 0.02			472.6 ± 0.3	
Average of TNB soil	**5.70 ± 0.05***	**5.78 ± 0.05***	**5.83 ± 0.06***	**465.7 ± 2.3***	**468.8 ± 3.7***

*values are mean ± standard error.

Table 12. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Weathered/Aged TNB Definitive Plant Toxicity Test

TNB Concentration ($mg\ kg^{-1}$)	pH - Alfalfa	pH - Japanese Millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	6.14	6.61	5.98	318.7 ± 8.6	334.4 ± 9.7	398.5 ± 36.3
Control (carrier)	6.30	6.62	5.96	354.2 ± 6.8	357.4 ± 9.1	351.4 ± 13.1
Average of controls	**6.22**	**6.62**	**5.97**	**336.5 ± 17.7[a]**	**345.9 ± 11.5[a]**	**374.9 ± 23.5[a]**
2		6.61	5.98		356.7 ± 10.2	286.8 ± 6.8
5	6.34	6.55		363.2 ± 8.5	326.8 ± 29.5	
10		6.45	5.93		332.4 ± 31.7	291.6 ± 3.5
20		6.50	5.87		319.1 ± 56.5	321.2 ± 11.2
40	6.45		6.02	314.2 ± 6.2		338.3 ± 16.8
60		6.55			290.2 ± 19.2	
80	6.46			287.4 ± 1.1		
120		6.45	5.95		329.4 ± 10.8	351.8 ± 14.5
160	6.46			282.9 ± 9.5		
250		6.04	5.75		292.5 ± 7.5	310.7 ± 20.6
320	6.29			286.8 ± 23.4		
600	6.32	6.14	5.73	305.8 ± 2.4	299.0 ± 7.8	311.9 ± 13.1
800	6.29			291.1 ± 9.6		
1200		5.93	5.73		296.2 ± 3.1	294.8 ± 10.4
1600	6.20			294.1 ± 8.0		
Average of TNB soil	**6.35 ± 0.03***	**6.36 ± 0.08***	**5.87 ± 0.04***	**303.2 ± 9.3***	**315.8 ± 7.6***	**313.4 ± 8.1***

*values are mean ± standard error.

Table 13. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Freshly Amended 2,4-DNT Definitive Plant Toxicity Test

2,4-DNT Nominal Concentration (mg kg^{-1})	pH - Alfalfa	pH - Japanese Millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	5.92	6.23	6.31	324.3 ± 17.9	421.6 ± 6.5	334.3 ± 19.9
Control (carrier)	6.07	6.26	6.41	330.2 ± 12.5	346.6 ± 18.8	361.8 ± 3.9
Average of controls	**6.00**	**6.25**	**6.36**	**327.2 ± 3.0***	**384.1 ± 37.5***	**348.1 ± 13.7***
1		6.41	6.37		395.0 ± 14.2	364.4 ± 8.0
2			6.56			379.9 ± 8.1
5	6.12	6.34	6.53	339.5 ± 6.0	392.2 ± 8.2	378.1 ± 11.8
10	6.23	6.41	6.54	304.5 ± 28.7	398.4 ± 4.0	376.7 ± 8.2
25	6.19	6.43	6.46	336.2 ± 11.3	376.8 ± 11.2	351.1 ± 20.3
50	6.12	6.26		347.6 ± 2.9	339.7 ± 35.1	
100	6.35	6.34	6.39	312.5 ± 6.4	324.7 ± 8.3	373.9 ± 6.8
300	6.17			297.9 ± 6.4		
600	6.17			301.0 ± 6.4		
Average of 2,4-DNT soil	**6.19 ± 0.03***	**6.37 ± 0.03***	**6.48 ± 0.03***	**319.89 ± 7.80***	**371.12 ± 12.83***	**370.68 ± 4.51***

*values are mean ± standard error.

Table 14. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Weathered/Aged 2,4-DNT Definitive Plant Toxicity Test

2,4-DNT Nominal Concentration (mg kg^{-1})	pH - Alfalfa	pH – Japanese Millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	6.38	6.37	6.07	305.6 ± 10.0	286.2 ± 30.5	340.2 ± 17.0
Control (carrier)	6.45	6.4	6.09	334.2 ± 11.4	303.6 ± 27.5	344.7 ± 12.2
Average of controls	**6.42**	**6.39**	**6.08**	**319.9 ± 14.3***	**294.9 ± 8.7***	**342.5 ± 2.2***
5		6.30	6.20		366.8 ± 7.3	361.9 ± 6.5
10	6.39		6.31	341.4 ± 6.1		374.0 ± 6.4
25	6.37	6.28	6.26	320.3 ± 9.0	345.9 ± 9.6	360.5 ± 11.1
50	6.35	6.29	6.30	328.8 ± 4.2	328.6 ± 5.5	308.2 ± 38.2
100	6.42	6.27	6.36	302.0 ± 10.2	313.5 ± 9.1	280.0 ± 10.2
200		6.23	6.24		289.2 ± 4.7	
300	6.40			300.3 ± 13.7		
600	6.38			279.8 ± 25.8		
1200	6.44			289.3 ± 8.2		
Average of 2,4-DNT soil	**6.39 ± 0.01***	**6.27 ± 0.01***	**6.28 ± 0.02***	**308.8 ± 8.4***	**328.8 ± 13.3***	**336.9 ± 18.2***

*values are mean ± standard error.

Table 15. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Freshly Amended 2,6-DNT Definitive Plant Toxicity Test

2,6-DNT Nominal Concentration (mg kg^{-1})	pH - Alfalfa	pH - Japanese Millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	5.9	5.9	5.6	358.4 ± 6.3	400.1 ± 4.9	345.7 ± 9.0
Control (carrier)	5.9	5.9	5.7	374.3 ± 11.1	420.0 ± 6.5	359.7 ± 7.5
Average of controls	**5.9**	**5.9**	**5.6**	**366.4 ± 7.9***	**410.1 ± 10.0***	**352.7 ± 7.0***
1	5.8			354.9 ± 5.9		
2	6.0			357.4 ± 16.8		
5	6.1	6.1	5.8	352.6 ± 7.1	419.0 ± 3.7	332.1 ± 24.1
10	6.0	6.0	5.8	353.1 ± 2.1	406.3 ± 6.5	334.4 ± 13.5
20	6.0	6.0	5.7	345.9 ± 7.5	381.4 ± 22.2	349.0 ± 18.9
40	6.1	5.9	5.8	368.3 ± 3.0	349.5 ± 4.5	366.6 ± 13.4
100	5.8	6.0	5.9	349.3 ± 2.3	358.4 ± 15.9	340.0 ± 13.0
500		5.9	5.8		350.9 ± 16.6	335.2 ± 11.9
Average of 2,6-DNT soil	**5.98 ± 0.04***	**5.98 ± 0.03***	**5.81 ± 0.02***	**354.50 ± 2.70***	**377.58 ± 12.14***	**342.88 ± 5.34***

*values are mean ± standard error.

Table 16. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Weathered/Aged 2,6-DNT Definitive Plant Toxicity Test

2,6-DNT Nominal Concentration (mg kg^{-1})	pH - Alfalfa	pH - Japanese Millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	6.11	6.26	6.03	301.8 ± 8.8	368.9 ± 3.5	356.7 ± 2.2
Control (carrier)	6.19	6.30	6.11	357.0 ± 14.9	312.5 ± 16.1	364.5 ± 4.3
Average of controls	**6.15**	**6.28**	**6.07**	**329.4 ± 27.6***	**340.7 ± 28.2***	**360.6 ± 3.9***
2	6.12			367.4 ± 2.6		
5	6.13			366.1 ± 12.8		
10	6.17	6.31	6.15	361.1 ± 8.4	361.6 ± 3.2	358.6 ± 3.0
20	6.08	6.31	6.10	367.3 ± 3.7	390.7 ± 1.9	375.4 ± 9.6
40	6.15	6.30	5.88	363.3 ± 8.9	346.7 ± 16.3	370.3 ± 2.9
50						
100	6.22	6.26	5.95	350.4 ± 8.5	340.5 ± 4.9	362.2 ± 4.4
150						
200	6.21			325.6 ± 3.6		
250						
300					304.3 ± 3.7	353.0 ± 1.1
500		6.11	5.93		300.3 ± 7.3	348.0 ± 11.3
1000		6.09	5.98		**340.7 14.1***	**361.3 ± 4.2***
Average of 2,6-DNT soil	**6.15 ± 0.02***	**6.23 ± 0.04***	**6.00 ± 0.04***	**357.3 ± 5.7***		

*values are mean ± standard error.

Table 17. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Freshly Amended RDX or HMX Definitive Plant Toxicity Test

Nominal Concentration ($mg\ kg^{-1}$)	pH - alfalfa	pH - Japanese millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	5.76	5.82	5.54	369.0 ± 10.3	427.5 ± 5.6	422.2 31.6
Control (carrier)	5.63	5.67	5.60	431.5 ± 9.3	416.5 ± 5.5	452.8 12.5
Average of controls	**5.70**	**5.75**	**5.57**	**400.3 ± 31.3***	**422.0 ± 5.5***	**437.5 ± 15.3***
RDX 10000	5.72	5.70	5.62	401.8 ± 7.7	382.9 ± 13.4	406.3 ± 3.6
HMX 10000	5.73	5.69	5.57	400.0 ± 3.4	382.7 ± 5.6	402.8 ± 2.8

*values are mean ± standard error.

Table 18. Measurement of Soil pH (n = 1) and Redox Potential (n = 3) at the End of Weathered/Aged RDX or HMX Definitive Plant Toxicity Test

Nominal Concentration ($mg\ kg^{-1}$)	pH - Alfalfa	pH - Japanese Millet	pH - Ryegrass	Redox Potential - Alfalfa	Redox Potential - Japanese Millet	Redox Potential - Ryegrass
Control (negative)	6.09	6.40	6.25	291.7 ± 11.8	288.1 ± 29.0	286.3 21.9
Control (carrier)	6.18	6.49	6.22	301.3 ± 10.0	297.2 ± 13.0	278.8 7.8
Average of controls	**6.14**	**6.45**	**6.24**	**296.5 ± 4.8***	**292.7 ± 4.6***	**282.6 ± 3.8***
RDX 10000	6.18	6.51	6.25	299.3 ± 10.6	333.2 ± 10.9	332.2 ± 5.6
HMX 10000	6.31	6.49	6.36	316.9 ± 30.6	336.8 ± 10.3	367.8 ± 6.6

*values are mean ± standard error.

Table 19. Comparisons of the Initial and Final Soil pH and Redox Values Determined in Definitive Phytotoxicity Tests

Compound Plant species	pH	Redox
Freshly amended TNB		
Alfalfa	Yes-	No
Japanese millet	Yes-	No
Ryegrass	No	No
Weathered/aged TNB		
Alfalfa	Yes+	Yes-
Japanese millet	Yes+	No
Ryegrass	No	No
Freshly amended 2,4-DNT		
Alfalfa	Yes+	Yes-
Japanese millet	Yes+	No
Ryegrass	Yes+	No
Weathered/aged 2,4-DNT		
Alfalfa	Yes+	No
Japanese millet	No	No
Ryegrass	No	Yes+
Freshly amended 2,6-DNT		
Alfalfa	Yes-	Yes-
Japanese millet	Yes-	Yes-
Ryegrass	Yes-	Yes-
Weathered/aged 2,6-DNT		
Alfalfa	Yes+	Yes+
Japanese millet	Yes+	Yes+
Ryegrass	No	Yes+
Freshly amended RDX and HMX		
Alfalfa	Yes-	Yes+
Japanese millet	Yes-	Yes+
Ryegrass	Yes-	Yes+
Weathered/aged RDX and HMX		
Alfalfa	Yes+	No
Japanese millet	Yes+	Yes+
Ryegrass	Yes+	Yes+

Yes+: Significant increase of pH or redox potential at the end of phytotoxicity test ($p < 0.05$).
Yes-: Significant decrease of pH or redox potential at the end of phytotoxicity test ($p < 0.05$).

3.3 EM Concentrations in Freshly Amended SSL Soil.

Concentrations of EMs in freshly amended soils were determined at the beginning (initial, T_0) and at the end (final, T_f) of each definitive toxicity test using acetonitrile and ATCLP extractions. Results of these analyses for each plant species test are presented in Tables 20 through 29. The initial percent recovery in freshly amended soils ranged from 84 to 110% for TNB; 86 to 103% for 2,4-DNT; 68 to 119% for 2,6-DNT; and 97 to 104% for RDX and HMX. Lower recovery values of 68 and 70% were observed for 2,6-DNT at concentrations of 2 and 20 mg kg^{-1}, respectively (Tables 26, 27, and 28).

The ATCLP extractable TNB, 2,4-DNT or 2,6-DNT concentrations increased proportionally with their nominal/acetonitrile concentrations (Tables 20 through 28). At concentrations below 100 mg kg^{-1}, ATCLP-based recovery ranged from 6 to 61% for TNB, 0 to 70% for 2,4-DNT, and 45 to 75% for 2,6-DNT. Higher ATCLP extractable values were determined in higher concentrations, ranging from 57 to 96% for TNB, 81 to 84% for 2,4-DNT, and 86% for 2,6-DNT. The ATCLP extractable concentrations of TNB, 2,4-DNT, and 2,6-DNT were below their water solubility level, which are 340, 280, and 206 mg L^{-1}, respectively (Hawari *et al.*, 2002). The RDX and HMX had low ATCLP-based recovery (Table 29). Only 2 and 0.2 % of RDX and HMX, respectively, were ATCLP extractable in soils freshly amended with 10 000 mg kg^{-1} RDX or HMX. These low ATCLP-based recoveries reflect the lower water solubility of both compounds, which were reported for RDX at 42 mg L^{-1} at 20 °C (Sikka *et al.*, 1980) and at 60 mg L^{-1} at 25 °C (Banerjee *et al.*, 1980). The water solubility of HMX was reported between 5 and 6.6 mg L^{-1} at 20 °C and 25 °C, respectively (Glover and Hoffsommer, 1973; McLellan *et al.*, 1992).

The presence of 3,5-DNA (3,5-dinitroaniline), a transformation product of TNB, was detected in every TNB treatment concentration in freshly amended SSL soil (data not shown). This suggests that some TNB was likely transformed at the beginning of these phytotoxicity tests. No transformation products or metabolites were detected at the beginning of the phytotoxicity tests with 2,4-DNT, 2,6-DNT, RDX, or HMX in freshly amended soil.

The percent decrease of EMs extracted by acetonitrile at the end (T_f) of each definitive test was calculated using the formula:

$$EM_{acetonitrile}\ \text{decrease}\ (\%) = 100 - (\text{Concentration at } T_f / \text{Concentration at } T_0 \times 100)$$

In freshly amended soil, percent decrease of either TNB, 2,4-DNT or 2,6-DNT was inversely related to their concentrations in acetonitrile extracts. At soil concentrations below 100 mg kg^{-1}, decrease in concentrations of TNB, 2,4-DNT, or 2,6-DNT ranged from 78 to 100%, 43 to 100%, and 39 to 100%, respectively. At concentrations above 100 mg kg^{-1}, decrease in concentrations of TNB, 2,4-DNT or 2,6-DNT ranged 0 to 52%, 19 to 24%, and from 21 to 24%, respectively. There was no significant decrease in acetonitrile extracted RDX in freshly amended soil in the 10000 mg kg^{-1} treatment, except for ryegrass where a 4% decrease was observed. In the 10000 mg kg^{-1} HMX treatment, acetonitrile extractable concentrations of HMX was decreased by 10, 17, and 13% in tests with alfalfa, millet, and ryegrass, respectively.

Text continues on page 41.

Table 20. Nominal and Measured TNB Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	5.1 ± 0.1	101	0.1 ± 0.1	99	1	12
40	39.2 ± 1.9	98	2.5 ± 0.2	94	18.2 ± 0.2	47
80	87.8 ± 0.9	110	19.6 ± 0.3	78	53.4 ± 1.8	61
160	170.9 ± 3.7	107	111.1 ± 6.3	35	122.8 ± 9.4	72
320	343 ± 13	107	286.7 ±2.4	16	301.7 ± 5.0	88
600	648 ± 14	108	587 ± 14	9	622.9 ± 6.5	96

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 21. Nominal and Measured TNB Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Japanese Millet

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
2	1.8 ± 0.0	91	0.0 ± 0.0	100	0.1 ± 0.0	6
5	5.1 ± 0.1	101	0.1 ± 0.1	98	0.6 ± 0.0	12
10	8.4 ± 0.1	84	0.36 ± 0.02	96	1.5 ± 0.1	18
20	21.5 ± 0.5	107	0.70 ±0.03	97	5.7 ± 0.2	27
60	64.1 ± 1.3	107	7.0 ± 0.6	89	33.8 ± 2.6	53
120	124.7 ± 4.4	104	59.5 ± 3.9	52	71.3 ± 1.3	57
250	220 ± 27	88	236 ± 11	0	191 ± 13	87
600	648 ± 14	108	587 ± 11	9	622.9 ± 6.5	96

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 22. Nominal and Measured TNB Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Ryegrass

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
2	1.8 ± 0.0	91	BDL	100	0.1 ± 0.0	6
10	8.4 ± 0.1	84	0.2 ± 0.1	98	1.5 ± 0.1	18
20	21.5 ± 0.5	107	0.53 ±0.04	98	5.7 ± 0.2	27
40	39.2 ± 1.9	98	2.3 ± 0.3	94	33.8 ± 2.6	47
120	124.7 ± 4.4	104	61.1 ± 8.0	51	71.3 ± 1.3	57
250	220 ± 27	88	231.6 ± 9.1	0	191.0 ±12.5	87
600	648 ± 14	108	605 ± 13	7	623 ± 7	96

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 23. Nominal and Measured 2,4-DNT Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	4.7 ± 0.1	95	0.37 ± 0.02	92	1.8 ± 0.1	39
10	9.1 ± 0.2	91	0.93 ± 0.03	90	3.8 ± 0.3	42
25	21.5 ± 0.2	86	4.9 ± 0.2	77	11.8 ± 0.8	55
50	46.5 ± 0.5	93	19.8 ± 1.0	57	11.8 ± 0.2	58
100	98.5 ± 1.3	99	53.8 ± 1.9	45	68.6 ± 2.0	70
300	278 ± 14	93	211.0 ± 7.0	24	225.9 ± 6.3	81
600	613 ± 43	102	496.1 ± 4.4	19	516.8 ± 3.2	84

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 24. Nominal and Measured 2,4-DNT Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Japanese Millet

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
1	1.0 ± 0.0	100	BDL	100	0.3 ± 0.0	25
5	4.7 ± 0.1	95	0.5 ± 0.02	90	1.8 ± 0.1	39
10	9.1 ± 0.2	91	1.3 ± 0.1	86	3.8 ± 0.3	42
25	21.5 ± 0.6	86	5.8 ± 0.7	73	11.8 ± 0.8	55
50	46.5 ± 0.5	93	21.4 ± 0.6	54	26.8 ± 0.2	58
100	98.5 ± 1.3	99	56.0 ± 3.3	43	68.6 ± 2.0	70

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 25. Nominal and Measured 2,4-DNT Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Ryegrass

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
1	1.0 ± 0.0	100	BDL	100	0.3 ± 0.0	30
2	2.1 ± 0.1	105	0.1 ± 0.0	94	0.7 ± 0.0	33
5	4.7 ± 0.1	94	0.5 ± 0.0	89	1.8 ± 0.1	38
10	9.1 ± 0.2	91	1.5 ± 0.04	84	3.8 ± 0.3	42
25	21.5 ± 0.6	86	8.3 ± 1.6	61	11.8 ± 0.8	55
50	46.5 ± 0.5	93	22.8 ± 1.4	51	26.8 ± 0.2	58
100	98.4 ± 1.3	98	51.6 ± 2.8	48	68.6 ± 1.9	70
Concentrations used for EC_{50}, EC_{20}, LOEC and NOEC final calculations						
0.5	0.5 ± 0.0	100	ND		BDL	BDL
1	0.9 ± 0.0	90	ND		0.3 ± 0.0	32
2.5	2.2 ± 0.1	88	ND		0.8 ± 0.0	36
5	3.8 ± 0.1	77	ND		1.4 ± 0.1	37
10	8.5 ± 0.1	85	ND		3.6 ± 0.1	43
20	17.1 ± 0.2	86	ND		8.8 ± 0.0	51
40	38.4 ± 0.6	96	ND		19.3 ± 0.7	50

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.
ND - Not determined.

Table 26. Nominal and Measured 2,6-DNT Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
1	1.2 ± 0.0	119	BDL	100	0.5 ± 0.0	46
2	1.4 ± 0.0	68	BDL	100	0.7 ± 0.0	52
5	4.1 ± 0.1	83	BDL	100	2.5 ± 0.2	60
10	8.0 ± 0.3	80	0.4 ± 0.3	95	4.3 ± 0.3	54
20	13.9 ± 0.6	70	4.6 ± 0.1	67	7.6 ± 0.2	55
40	29.7 ± 1.4	74	9.7 ± 0.2	67	22.0 ± 0.5	74
100	88.5 ± 1.7	89	53.8 ± 1.6	39	66.0 ± 2.0	75

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 27. Nominal and Measured 2,6-DNT Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Japanese Millet

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	4.1 ± 0.1	83	0.5 ± 0.1	89	2.5 ± 0.2	60
10	8.0 ± 0.3	80	1.2 ± 0.1	85	4.3 ± 0.3	54
20	13.9 ± 0.6	70	2.7 ± 0.1	81	7.6 ± 0.2	55
40	29.7 ± 1.4	74	16.9 ± 0.6	43	22.0 ± 0.5	74
100	88.5 ± 1.7	89	32.5 ± 2.1	63	66.0 ± 2.0	75
600	644.5 ± 6.8	107	489 ± 33	24	555.1 ± 4.8	86

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 28. Nominal and Measured 2,6-DNT Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Ryegrass

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	4.1 ± 0.1	83	0.1 ± 0.1	99	2.5 ± 0.2	60
10	8.0 ± 0.3	80	1.0 ± 0.1	88	4.3 ± 0.3	54
20	13.9 ± 0.6	70	4.0 ± 0.5	71	7.6 ± 0.2	55
40	29.7 ± 1.4	74	13.0 ± 0.9	56	22.0 ± 0.5	74
100	88.5 ± 1.7	89	40.5 ± 4.1	54	66.0 ± 2.0	75
600	644.5 ± 6.8	107	508 ± 61	21	555.1 ± 4.8	86

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 29. Nominal and Measured RDX or HMX Concentrations (mean ± S.E.; n = 3) in Freshly Amended SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa, Japanese Millet and Ryegrass

Nominal concentration (mg kg^{-1})	Acetonitrile extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL				BDL	
Control (carrier)	BDL				BDL	
RDX 10000 - alfalfa	9740 ± 150	97	10300 ± 180	0	197.8 ± 1.4	2
RDX 10000 - Japanese millet	9740 ± 150	97	10240 ± 110	0	197.8 ± 1.4	2
RDX 10000 - ryegrass	9740 ± 150	97	9370 ± 200	4	197.8 ± 1.4	2
HMX 10000 - alfalfa	10411 ± 810	104	9430 ± 380	10	18 ± 0.2	0.2
HMX 10000 - Japanese millet	10411 ± 810	104	8600 ± 210	17	18 ± 0.2	0.2
HMX 10000 - ryegrass	10411 ± 810	104	9060 ± 310	13	18 ± 0.2	0.2

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

3.4 EM Concentration in Weathered/Aged SSL Soil.

Weathering/aging of amended soils reduced concentrations of TNB, 2,4-DNT, and 2,6-DNT (Tables 30 through 38). Acetonitrile extraction-based recovery at concentrations below 100 mg kg^{-1} ranged from 0 to 28% for TNB, 25 to 37% for 2,4-DNT, and 12 to 20% for 2,6-DNT. At concentrations above 100 mg kg^{-1}, recovery ranged from 67 to 98% for TNB, 44 to 73% for 2,4-DNT, and 21 to 45% for 2,6-DNT, respectively. These data indicate that TNB was either strongly sorbed onto soil or was transformed at low concentrations (from 2 to 80 mg kg^{-1} nominal concentrations), and that TNB was more resistant to transformation or that the transformation process proceeded at relatively low rates at higher concentrations (from 120 to 1600 mg kg^{-1} nominal concentrations). Similarly, 2,4-DNT was strongly sorbed onto soil or was transformed at low concentrations (from 5 to 300 mg kg^{-1} nominal concentrations) and was more resistant to transformation or the transformation process proceeded at relatively low rates at nominal concentrations of 600-1200 mg kg^{-1}. Recovery of 2,6-DNT was below 45% at all tested concentrations, which indicated that a portion was likely sorbed onto soil. Concentrations of RDX or HMX remained stable in the 10000 mg kg^{-1} treatments with 95 and 93% recoveries respectively, following the 3-month weathering/aging period (Table 39).

Similar to the results in freshly amended soils, ATCLP extractable portions of TNB, 2,4-DNT, and 2,6-DNT in weathered/aged amended soils increased proportionally with EM concentrations. At nominal concentrations below 100 mg kg^{-1}, ATCLP-based recovery ranged from 0 to 31% for TNB, 27 to 53% for 2,4-DNT, and from 0 to 64% for 2,6-DNT. At nominal concentrations above 100 mg kg^{-1}, ATCLP-based recovery ranged from 48 to 93% for TNB, 55 to 81% for 2,4-DNT, and 68 to 89% for 2,6-DNT. In contrast with2,4-DNT and 2,6-DNT, which could be extracted using ATCLP method at concentrations as low as 5 mg kg^{-1}, TNB could not be extracted using this method at concentrations ranging from 2 to 20 mg kg^{-1}. Overall, ATCLP-based EM recoveries were significantly (Student's t test $p < 0.05$) lower in weathered/aged amended soil compared with freshly amended soil. The RDX and HMX were not appreciably transformed during weathering/aging procedure, and their ATCLP-based recoveries remained at 2 and 0.2% respectively, in weathered/aged amended soils (Table 39).

Transformation products detected in weathered/aged TNB and 2,4-DNT amended soils, suggest that these two EMs were in part transformed following exposure to sunlight and soil drying/wetting cycles. These transformation products included 3,5-DNA, 2-amino-4-nitrotoluene (2-A-4 NT), and 4-amino-2-nitrotoluene (4-A-2 NT). The 3,5-DNA was detected in all concentrations of weathered/aged TNB soil, but in greater amount at concentrations of 40, 60, and 80 mg kg^{-1}. Measurable amounts of 2-A-4 NT and 4-A-2 NT were detected in weathered/aged soil amended with 2,4-DNT at concentrations of 25, 50, and 200 mg kg^{-1}. There were no metabolites detected at the beginning of the phytotoxicity tests performed with 2,6-DNT, RDX, and HMX weathered/aged amended soils.

Text continues on page 47.

Table 30. Nominal and Measured TNB Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	BDL	0	BDL		BDL	0
40	2.1 ± 0.0	5	1 ± 0.1	65	0.5 ± 0.0	22
80	22.1 ± 0.4	28	11 ± 0.2	52	6.8 ± 0.7	31
160	114 ± 4	71	54 ± 2	53	66 ± 5	59
320	280 ± 10	88	271 ± 5	4	168 ± 1	59
600	580 ± 40	96	570 ± 15	2	430 ± 10	75
800	720 ± 10	90	710 ± 20	1	600 ± 20	83
1600	1560 ± 30	98	1530 ± 10	2	1460 ± 15	93

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 31. Nominal and Measured TNB Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Japanese Millet

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
2	BDL	0	BDL		BDL	0
5	BDL	0	0.1 ± 0.1		BDL	0
10	0.3 ± 0.0	3	0.1 ± 0.1	79	BDL	0
20	0.6 ± 0.0	3	0.1 ± 0.1	89	BDL	0
60	5 ± 0.2	9	1.3 ± 0.1	74	1.4 ± 0.1	27
120	81 ± 2	67	27 ± 1	66	39.1 ± 1.4	49
250	197 ± 7	79	187 ± 5	5	126 ± 1	64
600	575 ± 40	96	560 ± 20	3	430 ± 10	75
1200	984 ± 1	82	1160 ± 50	0	790 ± 3	80

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 32. Nominal and Measured TNB Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Ryegrass

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
2	BDL	0	BDL		BDL	0
10	0.3 ± 0.0	3	BDL	100	BDL	0
20	0.6 ± 0.0	3	BDL	100	BDL	0
40	2 ± 0.0	5	0.7 ± 0.0	65	0.5 ± 0.0	22
120	81 ± 2	67	30 ± 3	63	39 ± 1	49
250	197 ± 7	79	181 ± 8	8	126 ± 1	64
600	575 ± 40	96	520 ± 25	10	430 ± 10	75
1200	984 ± 1	82	1280 ± 90	0	790 ± 3	80

Table 33. Nominal and Measured 2,4-DNT Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
10	3.7 ± 0.2	37	2.8 ± 0.1	23	1.3 ± 0.1	35
25	7.8 ± 0.1	31	5.1 ± 0.1	34	2.8 ± 0.0	36
50	14.9 ± 0.3	30	15.8 ± 0.8	0	6.2 ± 0.3	42
100	32.1 ± 0.7	32	24.4 ± 0.6	24	16.9 ± 0.2	53
300	132 ± 3	44	128 ± 3	3	102 ± 3	78
600	353 ± 2	59	342 ± 9	3	270 ± 20	77
1200	880 ± 10	73	880 ± 30	0	710 ± 4	81
Concentrations used for EC_{50}, EC_{20}, LOEC and NOEC final calculations						
5	3.2 ± 0.1	64	ND		1.1 ± 0.1	34
10	6.2 ± 0.2	62	ND		2.2 ± 0.0	35
25	10.3 ± 0.5	41	ND		4.1 ± 0.1	40
50	25.2 ± 0.6	50	ND		11.5 ± 0.7	46
100	55.6 ± 2.3	56	ND		27.7 ± 0.3	50
150	89.2 ± 2.1	59	ND		47.6 ± 1.2	53
200	120.6 ± 4.4	60	ND		70.9 ± 0.5	59
250	153.4 ± 4.9	61	ND		104.6 ± 3.5	68

BDL - Below detection limit. HPLC detection limit = 0.5 mg kg^{-1} soil.
ND - Not determined.

Table 34. Nominal and Measured 2,4-DNT Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Japanese Millet

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	1.3 ± 0.0	25	1.1 ± 0.0	15	0.3 ± 0.0	27
10	3.7 ± 0.2	37	2.9 ± 0.1	23	1.3 ± 0.1	35
25	7.8 ± 0.1	31	5.7 ± 0.1	27	2.8 ± 0.0	36
50	14.9 ± 0.3	30	10.2 ± 0.3	32	6.2 ± 0.3	42
100	32.1 ± 0.7	32	24.9 ± 0.7	22	16.9 ± 0.2	53
200	90 ± 7	45	68 ± 1	25	50 ± 6	55

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 35. Nominal and Measured 2,4-DNT Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Ryegrass

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
5	1.3 ± 0.0	25	1.1 ± 0.1	15	0.3 ± 0.0	27
10	3.7 ± 0.2	37	3.0 ± 0.1	20	1.3 ± 0.1	35
25	7.8 ± 0.1	31	5.6 ± 0.2	27	2.8 ± 0.03	36
50	14.9 ± 0.3	30	12 ± 2	19	6.2 ± 0.3	42
100	32.1 ± 0.7	32	25.4 ± 0.1	21	16.9 ± 0.2	53
200	90 ± 7	45	73.9 ± 0.7	18	50 ± 6	55

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 36. Nominal and Measured 2,6-DNT Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
2	0.4 ± 0.1	19	0.1 ± 0.1	84	0.0 ± 0.0	0
5	0.6 ± 0.0	12	0.3 ± 0.0	52	0.2 ± 0.0	37
10	1.2 ± 0.0	12	0.8 ± 0.3	33	0.6 ± 0.1	48
20	3.3 ± 0.3	17	1.3 ± 0.1	61	1.5 ± 0.0	44
40	5.4 ± 0.1	13	2.7 ± 0.1	50	3.2 ± 0.1	59
100	14.9 ± 0.1	15	8.4 ± 0.4	44	9.5 ± 0.3	64
200	41.1 ± 0.8	21	25.0 ± 0.5	39	27.8 ± 0.2	68

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 37. Nominal and Measured 2,6-DNT Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Japanese Millet

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL	BDL	BDL	
Control (carrier)	BDL		BDL	BDL	BDL	
10	1.2 ± 0.0	12	BDL	100	0.6 ± 0.1	48
20	3.3 ± 0.3	17	1.6 ± 0.0	53	1.5 ± 0.0	44
40	5.4 ± 0.1	13	3.4 ± 0.1	38	3.2 ± 0.1	59
100	14.9 ± 0.1	15	8.9 ± 0.3	41	9.5 ± 0.3	64
500	139.5 ± 4.5	28	103.3 ± 3.2	26	104 ± 7	74
1000	447.3 ± 16.3	45	362.8 ± 14.5	19	397 ± 7	89

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 38. Nominal and Measured 2,6-DNT Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Ryegrass

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
50	7.5 ± 0.4	15	3.6 ± 0.1	52	3.6 ± 0.2	48
100	19.7 ± 0.6	20	9.4 ± 0.2	52	11.7 ± 0.8	59
150	37 ± 2	25	18.1 ± 0.6	51	23 ± 1	61
200	60 ± 2	30	33.3 ± 0.8	44	38 ± 1	63
250	75 ± 2	30	45 ± 2	40	54 ± 3	72
300	118 ± 4	39	69 ± 5	41	81 ± 3	69

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 39. Nominal and Measured RDX or HMX Concentrations (mean ± S.E.; n = 3) in Weathered/Aged SSL Soil Determined at the Beginning (T_0) and at the End (T_f) of Definitive Test with Alfalfa, Japanese Millet, and Ryegrass

Nominal Concentration (mg kg^{-1})	Acetonitrile Extraction at T_0 (mg kg^{-1})	Recovery at T_0 (%)	Acetonitrile Extraction at T_f (mg kg^{-1})	Decrease at T_f (%)	ATCLP Extraction at T_0 (mg kg^{-1})	ATCLP/ Acetonitrile (%)
Control (negative)	BDL		BDL		BDL	
Control (carrier)	BDL		BDL		BDL	
RDX 10000 - alfalfa	9500 ± 200	95	9500 ± 100	0	192 ± 1	2
RDX 10000 - Japanese millet	9500 ± 200	95	9100 ± 200	4	192 ± 1	2
RDX 10000 - ryegrass	9500 ± 200	95	9500 ± 200	0.1	192 ± 1	2
HMX 10000 - alfalfa	9300 ± 800	93	9800 ± 600	0	16 ± 0.1	0.2
HMX 10000 - Japanese millet	9300 ± 800	93	9000 ± 300	3	16 ± 0.1	0.2
HMX 10000 - ryegrass	9300 ± 800	93	9200 ± 400	2	16 ± 0.1	0.2

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

The decrease of TNB extracted by acetonitrile at the end of phytotoxicity tests (T_f) with weathered/aged amended soils exceeded 52% in treatments with nominal concentrations below 160 mg kg^{-1} but was small (< 10%) at concentrations above than 250 mg kg^{-1} for all three plant species (Tables 30, 31, and 32). The decrease of 2,4-DNT extracted by acetonitrile at T_f ranged from 0 to 34% and was not related to its nominal concentration (Tables 33, 34, and 35). The decrease of 2,6-DNT extracted by acetonitrile at T_f was inversely proportional to nominal soil concentrations. At concentrations below 100 mg kg^{-1}, the decrease in extractability by acetonitrile ranged from 44 to 84% for alfalfa, 41 to 100% for millet, and 52% for ryegrass. At concentrations above 100 mg kg^{-1}, the decrease in extractability by acetonitrile was 39% for alfalfa, 19 to 26% for millet, and 40 to 51% for ryegrass (Tables 36, 37, and 38).

Photolysis, microbial degradation, adsorption or fixation at binding sites within the soil and plant uptake are among possible mechanisms contributing to the decrease in concentrations of TNB, 2,4-DNT and 2,6-DNT in weathered/aged amended soils. The TNB was transformed in freshly and weathered/aged amended soils at concentrations below 100 mg kg^{-1}. At the higher concentrations, however, TNB was barely transformed in weathered/aged soil. Greater amounts of 3,5-DNA detected at TNB concentrations between 40 and 250 mg kg^{-1} support the TNB transformation hypothesis, although soil sorption at low concentrations cannot be discounted. The 2,4-DNT was more readily transformed in freshly amended soil than in weathered/aged 2,4-DNT amended soil. Some 2-A-4 NT and 4-A-2 NT were detected in 2,4-DNT weathered/aged amended soil at all concentrations, but none of these transformation products were detected at the concentration of 1200 mg kg^{-1}. At concentrations below 100 mg kg^{-1}, the decrease in extractability of 2,6-DNT by acetonitrile was similar in freshly amended and weathered/aged amended soils and was inversely proportional to nominal concentrations. There were no transformation products detected in 2,6-DNT amended soils, but soil sorption cannot be discounted.

There was no decrease in extractability of RDX by acetonitrile in weathered/aged amended soil in the 10000 mg kg^{-1} treatment except for the test with Japanese millet, where a 4% decrease in extractability by acetonitrile occurred. In the 10000 mg kg^{-1} HMX treatment, decrease in extractability by acetonitrile of 3 and 2% of HMX occurred in tests with Japanese millet and ryegrass, respectively. There were no metabolites detected in RDX and HMX amended soils. Analytical results show that RDX was not significantly transformed during the course of the phytotoxicity assays. The decrease in extractability by acetonitrile of HMX during the assays was greater in freshly amended soils compared with weathered/aged amended soils.

3.5 Range-Finding Plant Toxicity Tests.

To choose the best performing plants in the Sassafras sandy loam soil, different varieties of alfalfa, corn, Japanese millet, lettuce, and ryegrass were compared. The soil moisture condition was optimized and a level equivalent to 75% water holding capacity was chosen. This hydration produced better germination rates for most seeds. Kandy corn Canada No. 1 and Japanese millet seeds gave satisfactory germination with seedling emergence of 100% and 85%, respectively. Two varieties of perennial ryegrass (Cutter and Express) were compared. The Express variety gave slightly better results in the Sassafras soil with germination rate of 97% compared to 95% for the Cutter variety.

Alfalfa seeds were tested with and without inoculation of nitrogen-fixing bacteria. Germination rate of alfalfa (55% with bacterial inoculation and 62% without bacterial inoculation) was lower compared with the other species. Germination rate improved to 70% when lyophilized nitrogen-fixing bacteria were moisten and incubated at room temperature for 1 hr prior to inoculation onto alfalfa seeds,

Despite germinating and growing well in silica and OECD artificial soil, the Buttercrunch lettuce seeds germinated poorly in the Sassafras sandy loam soil when sown at a depth of few millimeters in the soil. Germination of six varieties of lettuce, including Buttercrunch, Grand Rapids, Boston Genecorp, Parris Island, Simpson Elite, and Green salad bowl, were tested. After 5 days of incubation, the Buttercrunch lettuce had the highest germination rate of 92%, compared to 48% for Grand Rapids, 82% for Boston Genecorp, 87% for Parris Island, 73% for Simpson Elite, and 5% for Green salad bowl variety.

Based on these results, Kandy Corn Canada No. 1, Japanese millet, Express perennial ryegrass, alfalfa inoculated with nitrogen-fixing bacteria and pre-incubated at room temperature, and Buttercrunch lettuce seeds were used for the range-finding tests to determine the three most sensitive species. Plants were exposed in triplicate to RDX, HMX, TNB, 2,4-DNT, and 2,6-DNT at concentrations of 1, 10, 100, 1000, and 10000 mg kg^{-1}.

During the range-finding tests and based on seeding emergence and shoot growth measurement endpoints (Table 40), no toxic effects were observed for RDX and HMX at concentrations of up to 10000 mg kg^{-1} for all five plants tested. The NOEC values were 9363 and 10373 mg kg^{-1} for RDX and HMX respectively, as derived from ANOVA.

The TNB, 2,4-DNT, and 2,6-DNT range-finding tests showed that these three energetic compounds affected the five plant species within test concentration ranges selected. Based on preliminary seedling emergence and growth EC_{20} values (Table 41), the three most sensitive species for TNB range-finding tests were Japanese millet, ryegrass, and lettuce. Based on seedling emergence and growth preliminary EC_{20} values (Table 42), the three most sensitive species range-finding testes were alfalfa, Japanese millet, and ryegrass. Corn and ryegrass showed very similar EC_{20} values for 2,6-DNT range-finding tests, therefore the four most sensitive species were alfalfa, corn, Japanese millet, and ryegrass, based on preliminary seedling emergence and growth EC_{20} values (Table 43).

On the basis of these range-finding tests, all plant species that were tested approved to be sensitive to 2,6-DNT, except for lettuce, which also showed high resistance to 2,4-DNT. Based on its response to TNB and 2,4-DNT, the second most resistant speices was corn. In addition, lettuce showed a poor germination rate in the carrier control, compared with the water control. Therefore, the three most sensitive species selected for use in the definitive tests were alfalfa, (dicotyledonous species), Japanese millet, and ryegrass, (which are monocotyledonous species).

Table 40. Summary of Ecotoxicological Parameters Determined from the Range-Finding Assays with RDX and HMX

Ecotoxicological Parameters	Alfalfa (mg kg^{-1})	Corn (mg kg^{-1})	Japanese Millet (mg kg^{-1})	Ryegrass (mg kg^{-1})	Lettuce (mg kg^{-1})
RDX					
LOEC-seedling emergence-T_{5-7d}	>9363	>9363	>9363	>9363	>9363
NOEC-seedling emergence-T_{5-7d}	9363	9363	9363	9363	9363
LOEC- Growth, fresh mass	>9363	>9363	>9363	>9363	>9363
NOEC- Growth, fresh mass	9363	9363	9363	9363	9363
LOEC- Growth, dry mass	>9363	>9363	>9363	>9363	>9363
NOEC- Growth, dry mass	9363	9363	9363	9363	9363
HMX					
LOEC-seedling emergence-T_{5-7d}	>10373	>10373	>10373	>10373	>10373
NOEC-seedling emergence-T_{5-7d}	10373	10373	10373	10373	10373
LOEC- Growth, fresh mass	>10373	>10373	>10373	>10373	>10373
NOEC- Growth, fresh mass	10373	10373	10373	10373	10373
LOEC- Growth, dry mass	>10373	>10373	>10373	>10373	>10373
NOEC- Growth, dry mass	10373	10373	10373	10373	10373

Table 41. Summary of Ecotoxicological Parameters Determined from the Range-Finding Assays with TNB

Ecotoxicological Parameters	Alfalfa (mg kg^{-1})	Corn (mg kg^{-1})	Japanese Millet (mg kg^{-1})	Ryegrass (mg kg^{-1})	Lettuce (mg kg^{-1})
EC_{50}-seedling emergence-T_{5-7d}	345	696	438	101	5854
EC_{20}-seedling emergence-T_{5-7d}	49	309	89	21	88
LOEC	116	1083	116	12	12
NOEC	12	116	12	<12	<12
EC_{50}- Growth, fresh mass	>116	114	53	99	61
EC_{20}- Growth, fresh mass	58	48	8	46	11
LOEC	116	116	12	116	12
NOEC	12	12	<12	12	<12
EC_{50}- Growth, dry mass	>116	428	94	116	82
EC_{20}- Growth, dry mass	69	59	42	53	22
LOEC	116	116	116	116	116
NOEC	12	12	12	12	12

Table 42. Summary of Ecotoxicological Parameters Determined from the Range-Finding

Assays with 2,4-DNT

Ecotoxicological Parameters	Alfalfa (mg kg^{-1})	Corn (mg kg^{-1})	Japanese Millet (mg kg^{-1})	Ryegrass (mg kg^{-1})	Lettuce (mg kg^{-1})
EC_{50}-seedling emergence-T_{5-7d}	443	84	52	52	3128
EC_{20}-seedling emergence-T_{5-7d}	130	40	24	27	304
LOEC	967	95	94	10	4897
NOEC	95	10	10	<10	967
EC_{50}- Growth, fresh mass	91	72	46	>10	55
EC_{20}- Growth, fresh mass	42	35	14	>10	16
LOEC	>95	95	10	10	10
NOEC	95	10	<10	<10	<10
EC_{50}- Growth, dry mass	>95	76	56	>10	71
EC_{20}- Growth, dry mass	67	36	22	>10	35
LOEC	>95	95	10	10	10
NOEC	95	10	<10	<10	<10

Table 43. Summary of Ecotoxicological Parameters Determined from the Range-Finding Assays with 2,6-DNT

Ecotoxicological Parameters	Alfalfa (mg kg^{-1})	Corn (mg kg^{-1})	Japanese Millet (mg kg^{-1})	Ryegrass (mg kg^{-1})	Lettuce (mg kg^{-1})
EC_{50}-seedling emergence-T_{5-7d}	65	78	279	249	1954
EC_{20}-seedling emergence-T_{5-7d}	32	37	58	39	401
LOEC	100	100	100	100	>4905
NOEC	10	10	10	10	4905
EC_{50}- Growth, fresh mass	34	68	54	83	64
EC_{20}- Growth, fresh mass	5	33	22	36	31
LOEC	100	100	100	100	10
NOEC	10	10	10	10	<10
EC_{50}- Growth, dry mass	44	71	56	83	69
EC_{20}- Growth, dry mass	7	34	26	39	25
LOEC	100	100	100	100	10
NOEC	10	10	10	10	<10

Neither RDX nor HMX were toxic to the five plant species in the range-finding tests (Table 43). Limit tests were performed with these two compounds, which included eight

replicates of 10 000 mg kg^{-1} HMX or RDX, eight replicates of control, eight replicates of carrier control, using freshly amended and weathered/aged amended SSL soil.

Boric acid positive controls were tested in triplicate. Concentrations were 175, 200, 230, 260, and 300 mg kg^{-1} for alfalfa; 65, 110, 175, 260, 345, and 460 mg kg^{-1} for Japanese millet; and 50, 80, 110, 150, and 200 mg kg^{-1} for ryegrass.

3.6 Definitive Plant Toxicity Tests.

Definitive plant toxicity tests were conducted to assess the effects of RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB on the terrestrial plant species alfalfa, Japanese millet, and ryegrass in freshly amended and weathered/aged SSL soil treatments. Measurement endpoints included germination (measured as the number of emerged seedlings) and growth (measured as fresh and dry shoot mass). These endpoints were assessed using 6 to 9 treatment concentrations that were determined from the range-finding studies (Tables 44 to 50). All ecotoxicological parameters were determined using measured chemical concentrations.

Germination in the negative and carrier (acetone) controls complied in all cases with quality control requirements. These were 70% for alfalfa and 85% for Japanese millet and ryegrass. Alfalfa germination in negative controls ranged from 68 to 76% and from 69 to 82% in carrier controls. Japanese millet germination in negative controls ranged from 83 to 96% and from 79 to 98% in carrier controls. Ryegrass germination in negative controls ranged from 85 to 94%, and from 75 to 95% in carrier controls.

Alfalfa fresh shoot mass ranged from 0.12 to 0.38 g in negative controls and from 0.27 to 0.45 g in carrier controls. Japanese millet had higher biomass, with fresh shoot mass ranging from 0.44 to 0.62 g in negative controls and from 0.39 to 0.63 g in carrier controls. Ryegrass fresh shoot mass was similar to alfalfa biomass, with fresh shoot mass ranging from 0.15 to 0.30 g in negative controls and from 0.13 to 0.34 g in carrier controls. Dry shoot mass was usually ten times lower than fresh shoot mass due to approximately 90% water content in plant shoot tissue.

The ecotoxicological parameters determined in this study included bounded NOEC/NOAEC and LOEC/LOAEC and EC_{20} and EC_{50} values. These parameters were determined for seedling emergence (shoot fresh and dry mass measurement endpoints). Measured concentrations from acetonitrile and ATCLP extractions were used in statistical analyses and parameter estimations. Coefficients of determinations (R^2) and ECp values were determined by nonlinear regression analyses using either logistic (Gompertz), logistic hormetic, or exponential models (Tables 51 to 63). The effect of weathering/aging of amended soils on EM toxicity for terrestrial plant species tested was determined by examining coefficients of determination from regression analyses performed to estimate shoot growth EC_{20} and EC_{50} values and their respective 95% confidence intervals. Data presented in Tables 64 to 69 identify EMs with a significant effect of the weathering/aging of amended soils for toxicity measurement endpoints used in the study.

Text continues on page 75.

Table 44. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Freshly Amended TNB in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Freshly Amended Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Error
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	14.0 (70%)	1.6	0.2432	0.0408	0.0245	0.0064
Control (carrier)	13.8 (69%)	1.2	0.3092	0.0280	0.0308	0.0021
5.1	13.8	1.1	0.2955	0.0352	0.0310	0.0034
39.2	14.5	0.7	0.2332	0.0122	0.0288	0.0017
87.8	14.8	0.8	0.1838	0.0071	0.0204	0.0011
170.9	7.0	2.5	0.0941	0.0256	0.0110	0.0035
343.4	0	0	0.0030	0	0.0003	0
647.8	0	0				
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	18.5 (93%)	0.3	0.5316	0.0115	0.0526	0.0008
Control (carrier)	16.8 (84%)	1.1	0.3946	0.0150	0.0426	0.0019
1.8	17.3	0.7	0.4214	0.0317	0.0434	0.0017
5.1	16.3	0.9	0.4455	0.0287	0.0485	0.0037
8.4	17.8	1.5	0.4007	0.0311	0.0477	0.0031
21.5	17.5	0.3	0.2563	0.0157	0.0378	0.0007
64.1	17.5	0.7	0.1657	0.0038	0.0319	0.0015
124.7	10.5	1.2	0.0688	0.0066	0.0122	0.0012
220.3	4.5	1.7	0.0227	0.0044	0.0047	0.0020
647.8	1	0	0.0016	0	0.0002	0
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	18.8 (94%)	0.5	0.3012	0.0131	0.0514	0.0018
Control (carrier)	15.0 (75%)	1.6	0.1947	0.0214	0.0301	0.0026
1.8	15.3	0.9	0.2137	0.0140	0.0278	0.0019
8.4	17.0	0.7	0.2505	0.0114	0.0373	0.0019
21.5	17.5	0.7	0.2080	0.0088	0.0336	0.0021
39.2	16.8	0.8	0.1902	0.0061	0.0298	0.0009
124.7	5.0	1.2	0.0663	0.0091	0.0127	0.0014
220.3	0	0	0.0058	0.0016	0.0008	0.0002
647.8	0	0	0.0031	0.0010	0.0004	0.0003

Table 45. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Weathered/Aged TNB in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Weathered/Aged Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Error
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	15.3 (76%)	1.6	0.3551	0.0757	0.0397	0.0088
Control (carrier)	15.6 (78%)	0.9	0.3489	0.0749	0.0346	0.0043
BDL	14.3	0.5	0.3402	0.0513	0.0355	0.0043
2.1	15.0	0.8	0.3563	0.0318	0.0315	0.0023
22.1	16.8	0.5	0.2643	0.0190	0.0318	0.0020
113.5	8.3	1.3	0.1104	0.0145	0.0128	0.0017
282.0	0	0	0.0024	0	0.0004	0
575.2	0	0				
722.0	0	0				
1564.1	0	0				
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	18.5 (93%)	0.9	0.5997	0.0393	0.0625	0.0035
Control (carrier)	19.5 (98%)	0.5	0.6029	0.0752	0.0604	0.0020
BDL	19.0	0.4	0.5724	0.0305	0.0549	0.0012
BDL	18.8	0.8	0.5077	0.0153	0.0564	0.0045
0.3	19.0	0.4	0.4435	0.0536	0.0500	0.0008
0.6	18.0	0.9	0.3676	0.0252	0.0457	0.0005
5.2	18.0	0.4	0.0593	0.0107	0.0197	0.0032
80.7	19.0	0	0.0503	0.0050	0.0118	0.0010
197.1	0	0				
575.2	0	0				
984.3	0	0				
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	18.8 (94%)	1.0	0.1734	0.0197	0.0364	0.0014
Control (carrier)	19.0 (95%)	0.7	0.2024	0.0290	0.0362	0.0016
BDL	18.5	0.9	0.1984	0.0073	0.0320	0.0014
0.3	20	0	0.2450	0.0166	0.0399	0.0007
0.6	19.3	0.5	0.2564	0.0221	0.0379	0.0017
2.1	17.0	0.4	0.2543	0.0139	0.0359	0.0018
80.7	14.8	2.1	0.1192	0.0067	0.0200	0.0023
197.1	0.5	0.5	0.0071	0.0024	0.0006	0.0004
575.2	0	0				
984.3	0	0				

BDL - Below detection limit. HPLC detection limit = 0.05 mg L^{-1} = 0.5 mg kg^{-1} soil.

Table 46. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Freshly Amended 2,4-DNT in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Freshly Amended Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Error
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	14.3 (71%)	0.8	0.1213	0.579	0.0152	0.0056
Control (carrier)	15.2 (76%)	0.4	0.2705	0.041	0.0282	0.0029
4.7	11.5	0.5	0.1739	0.012	0.0148	0.0018
9.1	13.5	1.1	0.1935	0.032	0.0181	0.0022
21.5	14.8	1.8	0.1833	0.015	0.0202	0.0007
46.5	14.5	0.7	0.1353	0.013	0.0139	0.0043
98.5	1.3	1.0	0.0099	0.002	0.0013	0.0004
278.1	0	0				
612.7	0	0				
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	18.0 (90%)	0.7	0.4636	0.0348	0.0418	0.0043
Control (carrier)	18.5 (93%)	0.3	0.4083	0.0271	0.0478	0.0024
1.0	19.0	0.4	0.3476	0.0245	0.0451	0.0027
4.7	17.3	0.9	0.2588	0.0197	0.0525	0.0018
9.1	17.3	0.7	0.2617	0.0159	0.0356	0.0015
21.5	16.8	0.5	0.0612	0.0054	0.0411	0.0021
46.5	16.0	0.4	0.0203	0.0030	0.0054	0.0010
98.5	0.8	0.8	0.0028	0.0019	0.0005	0.0004
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	18.5 (93%)	0.3	0.2680	0.0066	0.0362	0.0014
Control (carrier)	19.0 (95%)	0.4	0.2985	0.0140	0.0381	0.0021
0.5	19.0	0.7	0.2592	0.0079	0.0331	0.0015
0.9	19.0	0.4	0.2569	0.0074	0.0309	0.0009
2.2	19.0	0.4	0.3151	0.0126	0.0387	0.0019
3.8	19.5	0.3	0.3399	0.0112	0.0411	0.0020
8.5	18.8	0.8	0.3127	0.0111	0.0360	0.0018
17.1	8.3	1.5	0.0602	0.0155	0.0072	0.0021
38.4	0.3	0.3	0.0006	0.0004	0	0

Table 47. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Weathered/Aged 2,4-DNT in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Weathered/Aged Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Error
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	17.5 (88%)	1.9	0.4792	0.0446	0.0389	0.0021
Control (carrier)	16.2 (81%)	1.8	0.4843	0.0481	0.0370	0.0026
3.2	17.0	2.2	0.4479	0.0774	0.0383	0.0040
6.2	17.3	0.5	0.4394	0.0123	0.0401	0.0017
10.3	15.3	1.3	0.3235	0.0916	0.0313	0.0057
25.2	17.0	2.2	0.2437	0.0306	0.0227	0.0017
55.6	17.0	2.2	0.1795	0.0373	0.0191	0.0034
89.2	16.5	1.0	0.1435	0.0173	0.0169	0.0011
120.6	5.8	2.2	0.0403	0.0147	0.0042	0.0020
153.4	0.3	0.5	0.0030	0.0061	0.0002	0.0005
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	16.5 (83%)	0.3	0.4915	0.0169	0.0438	0.0013
Control (carrier)	15.8 (79%)	0.7	0.5030	0.0419	0.0450	0.0021
1.3	16.3	0.5	0.5040	0.0366	0.0469	0.0021
3.7	15.0	0.8	0.4039	0.0266	0.0456	0.0014
7.8	15.5	0.3	0.1858	0.0150	0.0315	0.0016
14.9	16.3	0.9	0.0380	0.0011	0.0097	0.0006
32.1	16.3	0.9	0.0146	0.0020	0.0055	0.0008
90.4	0	0				
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	17.0 (85%)	1.1	0.2964	0.0148	0.0401	0.0010
Control (carrier)	18.3 (90%)	0.5	0.3412	0.0070	0.0443	0.0006
1.3	18.0	0.7	0.3445	0.0185	0.0442	0.0010
3.7	18.8	0.5	0.3257	0.0194	0.0456	0.0016
7.8	15.8	1.1	0.0957	0.0153	0.0126	0.0013
14.9	0	0				
32.1	0	0				
90.4	0	0				

Table 48. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Freshly Amended 2,6-DNT in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Freshly Amended Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Error
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	13.5 (68%)	0.5	0.2079	0.0501	0.0308	0.0078
Control (carrier)	15.4 (77%)	1.6	0.3684	0.0806	0.0359	0.0058
1.2	15.0	1.0	0.3604	0.0019	0.0359	0.0003
1.4	15.3	1.2	0.3486	0.0356	0.0351	0.0024
4.1	15.0	1.2	0.1729	0.0606	0.0207	0.0041
8.0	17.0	0.4	0.2455	0.0561	0.0292	0.0052
13.9	11.0	1.4	0.0414	0.0129	0.0144	0.0044
29.7	4.5	1.0	0.0089	0.0030	0.0017	0.0004
88.5	0.3	0.3				
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	19.3 (96%)	0.3	0.4455	0.0103	0.0408	0.0008
Control (carrier)	18.3 (91%)	1.1	0.3893	0.0146	0.0457	0.0010
4.1	18.3	0.3	0.4645	0.0068	0.0522	0.0030
8.0	18.5	0.5	0.4773	0.0171	0.0423	0.0019
13.9	17.5	0.9	0.2865	0.0377	0.0331	0.0025
29.7	16.8	1.1	0.0209	0.0008	0.0092	0.0017
88.5	1.0	0.7	0.0012	0.0006	0.0006	0.0003
644.5	0	0				
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	18.3 (91%)	0.9	0.2011	0.0054	0.0342	0.0019
Control (carrier)	13.8 (94%)	0.3	0.1337	0.0072	0.0221	0.0008
4.1	18.5	0.3	0.1951	0.0075	0.0296	0.0023
8.0	18.0	0.6	0.1519	0.0145	0.0262	0.0007
13.9	17.8	0.5	0.1400	0.0233	0.0252	0.0014
29.7	11.8	0.8	0.1145	0.0089	0.0179	0.0006
88.5	0	0	0.0053	0.0027	0.0010	0.0006
644.5	0	0				

Table 49. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Weathered/Aged 2,6-DNT in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Weathered/Aged Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Eerror
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	13.5 (68%)	1.0	0.3853	0.0271	0.0071	0.0019
Control (carrier)	16.4 (82%)	1.1	0.4497	0.0279	0.0385	0.0025
0.4	16.3	1.5	0.4632	0.0426	0.0408	0.0023
0.6	15.8	0.8	0.4156	0.0123	0.0229	0.0032
1.2	11.8	1.4	0.2991	0.0455	0.0287	0.0035
3.3	14.5	1.5	0.3021	0.0862	0.0276	0.0062
5.4	13.5	1.2	0.2560	0.0212	0.0025	0.0031
14.9	12.3	1.1	0.1157	0.0348	0.0134	0.0029
41.1	9.0	1.1	0.0436	0.0066	0.0094	0.0015
Japanese Millet						
Control (negative)	19.0 (95%)	0.6	0.6171	0.0257	0.0345	0.0018
Control (carrier)	18.5 (93%)	0.4	0.6342	0.0241	0.0528	0.0013
1.2	17.0	0.4	0.5976	0.0227	0.0395	0.0038
3.3	17.8	1.0	0.5268	0.0104	0.0491	0.0014
5.4	17.8	0.3	0.4941	0.0151	0.0378	0.0020
14.9	18.0	0.9	0.1274	0.109	0.0135	0.0007
139.5	0	0				
447.3	0	0				
Ryegrass						
Control (negative)	17.8 (88%)	0.9	0.2897	0.0162	0.0397	0.0017
Control (carrier)	19.3 (94%)	0.5	0.3054	0.0121	0.0419	0.0022
7.5	19.5	0.3	0.3021	0.0035	0.0409	0.0005
19.7	18.8	0.3	0.2732	0.0136	0.0349	0.0005
37.2	16.8	1.1	0.1620	0.0079	0.0178	0.0008
59.7	6.8	0.8	0.0480	0.0084	0.0037	0.0011
75.3	1.0	0.6	0.0141	0.0054	0.0004	0.0003
117.7						

Table 50. Average (n = 4) Seedling Counts, Fresh Shoot Mass and Dry Shoot Mass of Alfalfa, Japanese Millet, and Ryegrass Exposed to Freshly Amended and Weathered/Aged RDX and HMX in Sassafras Sandy Loam Soils. Concentrations are based on acetonitrile extraction using USEPA Method 8330A. Twenty (20) seeds were sown in each replicate.

Concentration in Freshly Amended Soil (mg kg^{-1})	Seedling Counts	Standard Error	Fresh Shoot Mass (g)	Standard Error	Dry Shoot Mass (g)	Standard Error
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	14.3 (71%)	0.9	0.1743	0.0697	0.0208	0.0056
Control (carrier)	14.8 (74%)	1.1	0.3618	0.0293	0.0309	0.0031
RDX - 9740	14.9	0.7	0.3522	0.0520	0.0347	0.0039
HMX - 10411	15.1	0.7	0.3002	0.0444	0.0312	0.0036
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	18.5 (93%)	0.5	0.4809	0.0291	0.0489	0.0013
Control (carrier)	18.1 (91%)	0.5	0.3041	0.0216	0.0312	0.0014
RDX - 9740	17.6	0.4	0.4751	0.0260	0.0476	0.0026
HMX - 10411	17.6	0.2	0.4765	0.0198	0.0457	0.0015
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	18.7 (94%)	0.5	0.2600	0.0073	0.0340	0.0005
Control (carrier)	18.1 (91%)	0.3	0.2119	0.0042	0.0279	0.0006
RDX - 9740	18.8	0.3	0.2106	0.0041	0.0279	0.0004
HMX - 10411	19.0	0.4	0.2351	0.0066	0.0311	0.0008
Concentration in Weathered/Aged Soil (mg kg^{-1})	**Seedling Counts**	**Standard Error**	**Fresh Shoot Mass (g)**	**Standard Error**	**Dry Shoot Mass (g)**	**Standard Error**
Alfalfa	After 5 days		After 16 days		After 16 days	
Control (negative)	14.5 (73%)	1.0	0.2630	0.0725	0.0285	0.0030
Control (carrier)	12.8 (64%)	1.0	0.2431	0.0424	0.0255	0.0028
RDX - 9537	14.0	0.9	0.2587	0.0290	0.0274	0.0023
HMX - 9341	13.3	0.9	0.1569	0.0299	0.0247	0.0028
Japanese Millet	After 5 days		After 16 days		After 16 days	
Control (negative)	17.0 (85%)	0.7	0.1770	0.0239	0.0392	0.0025
Control (carrier)	17.9 (89%)	0.2	0.1698	0.0045	0.0364	0.0013
RDX - 9537	17.1	0.3	0.2284	0.0327	0.0395	0.0019
HMX - 9341	16.3	0.5	0.2658	0.0139	0.0378	0.0009
Ryegrass	After 7 days		After 19 days		After 19 days	
Control (negative)	18.0 (90%)	0.0	0.1500	0.0195	0.0304	0.0013
Control (carrier)	18.1 (91%)	0.4	0.1886	0.0179	0.0283	0.0018
RDX - 9537	17.9	0.8	0.2157	0.0093	0.0264	0.0023
HMX - 9341	18.3	0.5	0.3178	0.0178	0.0361	0.0012

Table 51. Effect of Freshly Amended Energetic Materials on Alfalfa in Sassafras Sandy Loam Soil (Acetonitrile Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9740*	10411*	88***	47	8
p			0.553	0.601	0.364
LOEC or LOAEC	>9740	>10411	171****	99	14
p			<0.0001	<0.0001	0.001
EC_{20}			145	>47	11
Confidence interval			69.3-220.9		6.0-15.9
EC_{50}			172	>47	19
Confidence interval			155.5-187.6		14.1-23.6
Model used (EC_{20})			H	H & G	G
R^2 (EC_{20})			0.967		0.956
Growth - Fresh mass					
NOEC	9740*	10411*	5*	<5	1.4
p			0.671		0.148
LOEC	>9740	>10411	39	5**	4
p or P(T<=t) two-tail	0.875	0.269	0.028	0.004	<0.0001
EC_{20}			38	11	1.3
Confidence interval			9.6-65.5	0-24.2	0-2.9
EC_{50}			107	38	5
Confidence interval			72.2-141.0	17.0-58.3	2.0-8.0
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.971	0.923	0.919
Growth - Dry mass					
NOEC	9740*	10411*	39	<5	1.4
p			0.556		0.351
LOEC	>9740	>10411	88	5**	4
p or P(T<=t) two-tail	0.468	0.953	0.007	0.001	0.001
EC_{20}			62	34	2.8
Confidence interval			27.8-96.0	9.7-59.1	0-6.1
EC_{50}			129	56	9.5
Confidence interval			96.5-161.4	32.9-79.4	4.3-14.6
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.972	0.902	0.935

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 52. Effect of Freshly Amended Energetic Materials on Alfalfa in Sassafras Sandy Loam Soil (ATCLP Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9740*	10411*	53***	27	4
p			0.553	0.601	0.364
LOEC or LOAEC	>9740	>10411	123****	69	8
p			<0.0001	<0.0001	0.001
EC_{20}			30	39	6
Confidence interval			15.7-45.2	9.9-68.8	2.3-9.6
EC_{50}			123	>27	12
Confidence interval			107.8-138.7		7.9-16.5
Model used (EC_{20})			H	H	G
R^2 (EC_{20})			0.899	0.975	0.953
Growth - Fresh mass					
NOEC	9740	10411	0.6*	<1.8	0.7
p			0.671		0.148
LOEC	>9740	>10411	18	1.8**	2.5
p or P(T<=t) two-tail	0.875	0.269	0.028	0.008	<0.0001
EC_{20}			18	10	0.7
Confidence interval			1.1-35.1	0-22.1	0-1.6
EC_{50}			68	27	3
Confidence interval			40.5-96.1	12.2-42.6	1.1-4.4
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.971	0.923	0.922
Growth - Dry mass					
NOEC	9740	10411	18	<1.8	0.7
p			0.556		0.351
LOEC	>9740	>10411	53	1.8**	2.5
p or P(T<=t) two-tail	0.468	0.953	0.007	0.001	0.001
EC_{20}			34	19	1
Confidence interval			10.3-57.6	2.6-34.6	0-3.06
EC_{50}			86	34	5
Confidence interval			58.9-113.6	16.7-51.5	2.3-8.2
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.972	0.901	0.939

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 53. Effect of Weathered/Aged Energetic Materials on Alfalfa in Sassafras Sandy Loam Soil (Acetonitrile Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9537*	9341*	22***	89***	5***
p			0.059	0.802	0.089
LOEC or LOAEC	>9537	>9341	114****	121****	15****
p			<0.0001	0.0006	0.018
EC_{20}			109	104	26
Confidence interval			107.1-111.6	91.0-116.6	0-127.5
EC_{50}			114	115	55
Confidence interval			---	108.7-121.0	8.8-100.2
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.989	0.989	0.971
Growth - Fresh mass					
NOEC or NOAEC	9537*	9341*	22	6	3
p			0.125	0.188	0.154
LOEC or LOAEC	>9537	>9341	114	10	5
p or P(T<=t) two-tail	0.766	0.120	<0.0001	<0.0001	<0.0001
EC_{20}			20	7	1.6
Confidence interval			0-48.9	2.0-11.1	0.1-3.2
EC_{50}			63	30	7
Confidence interval			19.3-107.4	20.1-40.3	3.7-10.6
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.930	0.976	0.962
Growth - Dry mass					
NOEC	9537*	9341*	22	6***	3
p			0.361	0.153	0.207
LOEC	>9537	>9341	114	10****	5
p or P(T<=t) two-tail	0.614	0.852	<0.0001	0.011	<0.0001
EC_{20}			46	15	0.4
Confidence interval			2.4-89.0	8.8-21.4	0-1.4
EC_{50}			92	42	5
Confidence interval			58.8-125.0	28.5-55.9	0-10.6
Model used (EC_{20})			G	H	G
R^2 (EC_{20})			0.966	0.979	0.911

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 54. Effect of Weathered/Aged Energetic Materials on Alfalfa in Sassafras Sandy Loam Soil (ATCLP Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9537*	9341*	7***	48***	3***
p			0.059	0.802	0.060
LOEC or LOAEC	>9537	>9341	67****	71****	10****
p			<0.0001	0.0006	0.009
EC_{20}			64	36	4
Confidence interval			63.3-65.5	17.1-54.9	0-13.3
EC_{50}			67	66	41
Confidence interval			---	61.7-71.1	5.3-76.0
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.989	0.981	0.972
Growth - Fresh mass					
NOEC	9537*	9341*	7	2	2
p			0.125	0.188	0.086
LOEC	>9537	>9341	67	4	3
p or P(T<=t) two-tail	0.766	0.120	<0.0001	<0.0001	<0.0001
EC_{20}			7	2	0.7
Confidence interval			0-19.3	0.5-4.3	0-1.3
EC_{50}			29	14	4
Confidence interval			1.4-56.6	8.7-19.3	2.0-5.9
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.929	0.977	0.966
Growth - Dry mass					
NOEC or NOAEC	9537*	9341*	7	2***	1
p			0.361	0.153	0.095
LOEC or LOAEC	>9537	>9341	67	4****	3
p or P(T<=t) two-tail	0.614	0.852	<0.0001	0.011	<0.0001
EC_{20}			22	6	0.1
Confidence interval			0-49.2	3.4-9.1	0-0.2
EC_{50}			51	20	2
Confidence interval			27.0-75.4	12.7-27.8	0-4.5
Model used (EC_{20})			G	H	G
R^2 (EC_{20})			0.966	0.980	0.929

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 55. Effect of Freshly Amended Energetic Materials on Japanese Millet in Sassafras Sandy Loam Soil (Acetonitrile Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC	9740*	10411*	64	9	30
p			0.721	0.141	0.211
LOEC	>9740	>10411	125	22	89
p			0.001	0.044	<0.0001
EC_{20}			109	55	40
Confidence interval			74.0-144.2	46.4-62.7	28.3-51.6
EC_{50}			204	70	57
Confidence interval			167.8-239.2	62.7-77.9	46.0-67.6
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.988	0.994	0.992
Growth - Fresh mass					
NOEC or NOAEC	9740*	10411*	8***	1	<4
p			0.837	0.019	
LOEC or LOAEC	>9740	>10411	22****	5	4**
p or P(T<=t) two-tail	0.0002	0.00004	<0.0001	<0.0001	0.009
EC_{20}	stimulation	stimulation	16	3.5	13
Confidence interval			11.5-21.1	1.6-5.4	12.3-14.3
EC_{50}			36	10	16
Confidence interval			26.7-44.6	7.6-13.1	14.8-17.8
Model used (EC_{20})			H	G	H
R^2 (EC_{20})			0.984	0.975	0.991
Growth - Dry mass					
NOEC	9740*	10411*	22	5	8***
p			0.118	0.083	0.225
LOEC	>9740	>10411	64	9	14****
p or P(T<=t) two-tail	0.0002	0.000006	0.001	<0.0001	<0.0001
EC_{20}	stimulation	stimulation	43	25	11
Confidence interval			26.5-58.7	17.6-32.7	9.4-13.4
EC_{50}			89	34	18
Confidence interval			73.3-104.3	28.4-40.3	15.5-20.1
Model used (EC_{20})			G	G	H
R^2 (EC_{20})			0.985	0.978	0.989

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 56. Effect of Freshly Amended Energetic Materials on Japanese Millet in Sassafras Sandy Loam Soil (ATCLP Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC	9740*	10411*	34	4	22
p			0.721	0.141	0.211
LOEC	>9740	>10411	71	12	66
p			0.001	0.044	<0.0001
EC_{20}			63	33	30
Confidence interval			31.7-95.1	26.5-38.5	21.0-38.8
EC_{50}			168	45	43
Confidence interval			119.6-216.5	38.8-50.8	34.3-50.6
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.987	0.994	0.992
Growth - Fresh mass					
NOEC or NOAEC	9740*	10411*	1.5***	<0.3	<3
p			0.837		
LOEC or LOAEC	>9740	>10411	6****	0.3**	3**
p or P(T<=t) two-tail	0.0002	0.00004	<0.0001	0.019	0.009
EC_{20}	stimulation	stimulation	3	1	7
Confidence interval			2.0-4.7	0.5-2.1	6.6-7.9
EC_{50}			11	5	9
Confidence interval			6.5-15.1	3.3-6.1	8.0-10.3
Model used (EC_{20})			H	G	H
R^2 (EC_{20})			0.983	0.977	0.991
Growth - Dry mass					
NOEC or NOAEC	9740*	10411*	6***	2	4***
p			0.118	0.083	0.225
LOEC or LOAEC	>9740	>10411	34****	4	8****
p or P(T<=t) two-tail	0.0002	0.000006	0.001	<0.0001	<0.0001
EC_{20}	stimulation	stimulation	10	14	6
Confidence interval			4.4-15.5	9.7-19.1	5.1-7.4
EC_{50}			49	20	11
Confidence interval			40.5-57.5	16.0-23.4	8.8-12.2
Model used (EC_{20})			H	G	H
R^2 (EC_{20})			0.976	0.978	0.99

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 57. Effect of Weathered/Aged Energetic Materials on Japanese Millet in Sassafras Sandy Loam Soil (Acetonitrile Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9537*	9341*	81	32***	15***
p			0.374	0.584	0.581
LOEC or LOAEC	>9537	>9341	197	90****	140****
p			<0.0001	<0.0001	<0.0001
EC_{20}			139	>32	>15
Confidence interval			0-294.3	---	
EC_{50}			163	86	>15
Confidence interval			63.7-262.4		
Model used (EC_{20})			G	H	H & G
R^2 (EC_{20})			0.992	0.994	
Growth - Fresh mass					
NOEC	9537*	9341*	<0.3	1.3*	1
p				0.977	0.125
LOEC	>9537	>9341	0.3**	4	3
p or P(T<=t) two-tail	0.119	0.0002	0.017	0.015	<0.0001
EC_{20}		stimulation	0.3	3.5	4.8
Confidence interval			0.14-0.44	2.3-4.6	3.9-5.8
EC_{50}			0.9	6.5	9
Confidence interval			0.4-1.4	5.4-7.5	8.4-10.4
Model used (EC_{20})			E	G	G
R^2 (EC_{20})			0.972	0.982	0.995
Growth - Dry mass					
NOEC	9537*	9341*	<0.3	4	3
p				0.802	0.184
LOEC	>9537	>9341	0.3**	8	5
p or P(T<=t) two-tail	0.200	0.393	0.013	<0.0001	<0.0001
EC_{20}			0.7	6	6
Confidence interval			0.39-0.91	4.9-7.7	3.1-8.5
EC_{50}			2	10	11
Confidence interval			1.2-2.8	9.1-11.5	8.3-13.2
Model used (EC_{20})			E	G	G
R^2 (EC_{20})			0.990	0.989	0.979

G: Gompertz model
H: Hormetic model
E: Exponential model

* Unbounded NOEC
** Unbounded LOEC

*** NOAEC
**** LOAEC

Table 58. Effect of Weathered/Aged Energetic Materials on Japanese Millet in Sassafras Sandy Loam Soil (ATCLP Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC	9537*	9341*	39	17	10***
p			0.622	0.584	0.581
LOEC	>9537	>9341	126	50	104****
p			<0.0001	<0.0001	<0.0001
EC_{20}			80	>17	3
Confidence interval			0-196		2.8-3.6
EC_{50}			98	>17	53
Confidence interval			20-176		---
Model used (EC_{20})			G	G	H
R^2 (EC_{20})			0.992		0.935
Growth - Fresh mass					
NOEC	9537*	9341*	<1.4	0.3*	0.6*
p				0.977	0.125
LOEC	>9537	>9341	1.4**	1.3	1.5
p or P(T<=t) two-tail	0.119	0.0002	<0.0001	0.015	<0.0001
EC_{20}		stimulation	0.1	1.2	3
Confidence interval			0-0.33	0.7-1.6	2.1-3.6
EC_{50}			0.3	2.3	6
Confidence interval			0-1.03	1.9-2.7	5.0-6.5
Model used (EC_{20})			E	G	G
R^2 (EC_{20})			0.948	0.982	0.994
Growth - Dry mass					
NOEC	9537*	9341*	<1.4	1	1
p				0.802	0.184
LOEC	>9537	>9341	1.4**	3	3
p or P(T<=t) two-tail	0.200	0.393	<0.0001	<0.0001	<0.0001
EC_{20}			0.2	2	3
Confidence interval			0.1-0.33	1.6-2.8	1.5-4.8
EC_{50}			0.7	4	6
Confidence interval			0.3-1.0	3.5-4.6	4.8-8.0
Model used (EC_{20})			E	G	G
R^2 (EC_{20})			0.983	0.989	0.980

G: Gompertz model
H: Hormetic model
E: Exponential model

* Unbounded NOEC
** Unbounded LOEC

*** NOAEC
**** LOAEC

Table 59. Effect of Freshly Amended Energetic Materials on Ryegrass in Sassafras Sandy Loam Soil (Acetonitrile Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9740*	10411*	39***	9***	<4
p			0.192	0.803	
LOEC or LOAEC	>9740	>10411	125****	17****	4**
p			<0.0001	<0.0001	<0.0001
EC_{20}			28	8	29
Confidence interval			1.4-55.4	6.9-9.2	25.5-31.7
EC_{50}			95	16	38
Confidence interval			76.6-114.1	15.2-17.3	33.0-43.8
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.958	0.995	0.992
Growth - Fresh mass					
NOEC or NOAEC	9740*	10411*	39***	2***	30***
p			0.783	0.295	0.294
LOEC or LOAEC	>9740	>10411	125****	4****	89****
p or P(T<=t) two-tail	0.0005	<0.0001	<0.0001	0.013	<0.0001
EC_{20}	stimulation	stimulation	45	11	18
Confidence interval			35.1-55.7	10.2-12.0	4.4-31.7
EC_{50}			75	13	39
Confidence interval			59.2-91.1	12.2-14.6	19.1-59.1
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.981	0.991	0.944
Growth - Dry mass					
NOEC or NOAEC	9740*	10411*	39***	9***	14***
p			0.892	0.366	0.085
LOEC or LOAEC	>9740	>10411	125****	17****	30****
p or P(T<=t) two-tail	<0.0001	<0.0001	<0.0001	<0.0001	0.026
EC_{20}	stimulation	stimulation	56	11	26
Confidence interval			42.9-67.3	9.5-11.7	21.0-31.8
EC_{50}			89	13	39
Confidence interval			69.5-108.7	11.6-14.5	30.8-46.2
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.980	0.987	0.984

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 60. Effect of Freshly Amended Energetic Materials on Ryegrass in Sassafras Sandy Loam Soil (ATCLP Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC or NOAEC	9740*	10411*	18***	4***	<3
p			0.192	0.769	
LOEC or LOAEC	>9740	>10411	71****	9****	3**
p			<0.0001	<0.0001	<0.0001
EC_{20}			32	3	21
Confidence interval			23.3-39.9	2.6-4.2	19.0-23.5
EC_{50}			49	8	28
Confidence interval			38.1-60.1	7.6-8.9	23.2-32.1
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.985	0.995	0.991
Growth - Fresh mass					
NOEC or NOAEC	9740*	10411*	18***	0.3*	22***
p			0.783	0.151	0.294
LOEC or LOAEC	>9740	>10411	71****	0.8	66****
p or P(T<=t) two-tail	0.0005	<0.0001	<0.0001	0.022	<0.0001
EC_{20}	stimulation	stimulation	20	5	20
Confidence interval			9.1-30.8	4.5-5.5	11.3-28.0
EC_{50}			46	6	29
Confidence interval			30.4-61.5	5.6-7.1	8.1-49.0
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.970	0.991	0.955
Growth - Dry mass					
NOEC or NOAEC	9740*	10411*	18***	4***	8***
p			0.892	0.873	0.085
LOEC or LOAEC	>9740	>10411	71****	9****	22****
p or P(T<=t) two-tail	<0.0001	<0.0001	<0.0001	<0.0001	0.026
EC_{20}	stimulation	stimulation	27	5	20
Confidence interval			19.0-34.8	4.1-5.3	15.2-24.2
EC_{50}			49	6	28
Confidence interval			35.6-62.0	5.3-7.0	22.4-34.0
Model used (EC_{20})			H	H	H
R^2 (EC_{20})			0.979	0.987	0.983

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 61. Effect of Weathered/Aged Energetic Materials on Ryegrass in Sassafras Sandy Loam Soil (Acetonitrile Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC	9537*	9341*	81	4	20
p			0.172	0.586	0.547
LOEC	>9537	>9341	197	8	37
p			<0.0001	0.014	0.006
EC_{20}			107	>8	42
Confidence interval			81.1-133.4		38.0-45.3
EC_{50}			150	>8	54
Confidence interval			131-168		51.9-56.4
Model used (EC_{20})			G	H & G	G
R^2 (EC_{20})			0.992		0.995
Growth - Fresh mass					
NOEC	9537*	9341*	<0.3	4	8*
p			0.034	0.498	0.631
LOEC	>9537	>9341	0.3**	8	20
p or P(T<=t) two-tail	0.201	0.0002	<0.0001	<0.0001	0.022
EC_{20}		stimulation	46	5	24
Confidence interval			12.6-78.4	3.6-6.8	20.5-27.0
EC_{50}			83	7	39
Confidence interval			61.1-104.4	5.9-7.5	36.0-41.3
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.969	0.992	0.994
Growth - Dry mass					
NOEC or NOAEC	9537*	9341*	2	4***	8*
p			0.366	0.421	0.974
LOEC or LOAEC	>9537	>9341	81	8****	20
p or P(T<=t) two-tail	0.541	0.003	<0.0001	<0.0001	<0.0001
EC_{20}		stimulation	51	2	21
Confidence interval			29.6-72.2	0-4.0	18.2-23.3
EC_{50}			86	8	34
Confidence interval			73.6-99.0	---	31.9-36.0
Model used (EC_{20})			G	H	G
R^2 (EC_{20})			0.989	0.990	0.995

G: Gompertz model * Unbounded NOEC *** NOAEC
H: Hormetic model ** Unbounded LOEC **** LOAEC

Table 62. Effect of Weathered/Aged Energetic Materials on Ryegrass in Sassafras Sandy Loam Soil (ATCLP Extraction; n = 3)

Ecotoxicological Parameters (n = 4)	RDX (mg kg^{-1})	HMX (mg kg^{-1})	TNB (mg kg^{-1})	2,4-DNT (mg kg^{-1})	2,6-DNT (mg kg^{-1})
Germination - T 5d					
NOEC	9537*	9341*	0.5	1.3	12
p			0.148	0.586	0.547
LOEC	>9537	>9341	39	3	23
p			0.05	0.014	0.006
EC_{20}			57	3	25
Confidence interval			38.6-74.8	2.8-2.9	22.2-27.4
EC_{50}			88	>3	34
Confidence interval			73.2-102		32.3-35.5
Model used (EC_{20})			G	G	G
R^2 (EC_{20})			0.992	0.995	0.995
Growth - Fresh mass					
NOEC or NOAEC	9537*	9341*	0.5***	1	4*
p			0.179	0.498	0.631
LOEC or LOAEC	>9537	>9341	39****	3	12
p or P(T<=t) two-tail	0.201	0.0002	<0.0001	<0.0001	0.022
EC_{20}		stimulation	33	1.8	14
Confidence interval			20.0-45.2	1.3-2.4	11.9-16.2
EC_{50}			40	2.4	24
Confidence interval			34.1-46.0	2.1-2.7	22.0-25.5
Model used (EC_{20})			H	G	G
R^2 (EC_{20})			0.971	0.992	0.993
Growth - Dry mass					
NOEC	9537*	9341*	0.5	1***	4*
p			0.785	0.421	0.974
LOEC	>9537	>9341	39	3****	12
p or P(T<=t) two-tail	0.541	0.003	<0.0001	<0.0001	<0.0001
EC_{20}			21	>1.3	12
Confidence interval			9.7-33.1		10.7-13.9
EC_{50}			43	>1.3	21
Confidence interval			34.5-51.0		19.4-22.0
Model used (EC_{20})			G	G & H	G
R^2 (EC_{20})			0.989		0.995

G: Gompertz model
H: Hormetic model
* Unbounded NOEC
** Unbounded LOEC
*** NOAEC
**** LOAEC

Table 63. Summary of Coefficients of Determination (R^2) for Acetonitrile and ATCLP Extractable Measures of Exposure Determined by Nonlinear Regressions for Plant Measurement Endpoints (EC_{20} Levels) in Definitive Toxicity Tests of Energetic Materials in Freshly Amended and Weathered/Aged Amended SSL Soil

Compound	Seedling Emergence		Shoot Fresh Mass		Shoot Dry Mass	
Plant Species	Acetonitrile	ATCLP	Acetonitrile	ATCLP	Acetonitrile	ATCLP
Freshly Amended TNB						
Alfalfa	0.967	0.899	0.971	0.971	0.972	0.972
Japanese millet	0.988	0.987	0.984	0.983	0.985	0.976
Ryegrass	0.958	0.985	0.981	0.970	0.980	0.979
Weathered/Aged TNB						
Alfalfa	0.989	0.989	0.930	0.929	0.966	0.966
Japanese millet	0.992	0.992	0.972	0.948	0.990	0.983
Ryegrass	0.992	0.992	0.969	0.971	0.989	0.989
Freshly Amended 2,4-DNT						
Alfalfa	ND	0.975	0.923	0.923	0.902	0.901
Japanese millet	0.994	0.994	0.975	0.977	0.978	0.978
Ryegrass	0.995	0.995	0.991	0.991	0.987	0.987
Weathered/Aged 2,4-DNT						
Alfalfa	0.989	0.981	0.976	0.977	0.979	0.980
Japanese millet	0.994	ND	0.982	0.982	0.989	0.989
Ryegrass	ND	0.995	0.992	0.992	0.990	ND
Freshly Amended 2,6-DNT						
Alfalfa	0.956	0.953	0.919	0.922	0.935	0.939
Japanese millet	0.992	0.992	0.991	0.991	0.989	0.990
Ryegrass	0.992	0.991	0.944	0.955	0.984	0.983
Weathered/Aged 2,6-DNT						
Alfalfa	0.971	0.972	0.962	0.966	0.911	0.929
Japanese millet	ND	0.935	0.995	0.994	0.979	0.980
Ryegrass	0.995	0.995	0.994	0.993	0.995	0.995

ND - not determined

Table 64. Effect of Weathering/Aging (W/A) of Amended Soil on Toxicity of Nitroaromatic Energetic Materials for Alfalfa Using Acetonitrile Extraction

	Fresh TNB	W/A TNB	Fresh 2,4-DNT	W/A 2,4-DNT	Fresh 2,6-DNT	W/A 2,6-DNT
Growth - Fresh Mass						
EC_{20}	38	20	11	7	1.3	1.6
Confidence interval	10-66	0-49	0-24	2-11	0-3	0.1-3
Significant difference	no		no		no	
EC_{50}	107	63	38	30	5	7
Confidence interval	72-141	19-107	17-58	20-40	2-8	4-11
Significant difference	no		no		no	
Growth - Dry Mass						
EC_{20}	62	46	34	15	2.8	0.4
Confidence interval	28-96	2-89	10-59	9-21	0-6	0-1
Significant difference	no		no		no	
EC_{50}	129	92	56	42	9.5	5
Confidence interval	97-161	59-125	33-79	29-56	4-15	0-11
Significant difference	no		no		no	

Table 65. Effect of Weathering/Aging (W/A) of Amended Soil on Toxicity of Nitroaromatic Energetic Materials for Alfalfa Using ATCLP Extraction

	Fresh TNB	W/A TNB	Fresh 2,4-DNT	W/A 2,4-DNT	Fresh 2,6-DNT	W/A 2,6-DNT
Growth - Fresh Mass						
EC_{20}	18	7	10	2	0.7	0.7
Confidence interval	1-35	0-19	0-22	0.5-4.3	0-1.6	0-1.3
Significant difference	no		no		no	
EC_{50}	68	29	27	14	3	4
Confidence interval	41-96	1-57	12-43	9-19	1-4	2-6
Significant difference	no		no		no	
Growth - Dry Mass						
EC_{20}	34	22	19	6	1	0.1
Confidence interval	10-58	0-49	3-35	3-9	0-3	0-0.2
Significant difference	no		no		no	
EC_{50}	86	51	34	20	5	2
Confidence interval	59-114	27-75	17-52	13-28	2-8	0-5
Significant difference	no		no		no	

Table 66. Effect of Weathering/Aging (W/A) of Amended Soil on Toxicity of Nitroaromatic Energetic Materials for Japanese Millet Using Acetonitrile Extraction

	Fresh TNB	W/A TNB	Fresh 2,4-DNT	W/A 2,4-DNT	Fresh 2,6-DNT	W/A 2,6-DNT
Growth - Fresh Mass						
EC_{20}	16	0.3	3.5	3.5	13	4.8
Confidence interval	12-21	0.1-0.5	1.6-5.4	2.3-4.6	12-14	4-6
Significant difference	**yes**		no		**yes**	
EC_{50}	36	0.9	10	6.5	16	9
Confidence interval	27-45	0.4-1.4	7.6-13.1	5.4-7.5	15-18	8-10
Significant difference	**yes**		**yes**		**yes**	
Growth - Dry Mass						
EC_{20}	43	0.7	25	6	11	6
Confidence interval	27-59	0.4-0.9	18-33	5-8	9.4-13.4	3.1-8.5
Significant difference	**yes**		**yes**		**yes**	
EC_{50}	89	2	34	10	18	11
Confidence interval	73-104	1-3	28-40	9-12	16-20	8-13
Significant difference	**yes**		**yes**		**yes**	

yes - weathering/aging process significantly increased toxicity.

Table 67. Effect of Weathering/Aging (W/A) of Amended Soil on Toxicity of Nitroaromatic Energetic Materials for Japanese Millet Using ATCLP Extraction

	Fresh TNB	W/A TNB	Fresh 2,4-DNT	W/A 2,4-DNT	Fresh 2,6-DNT	W/A 2,6-DNT
Growth - Fresh Mass						
EC_{20}	3	0.1	1	1.2	7	3
Confidence interval	2-5	0-0.33	0.5-2.1	0.7-1.6	7-8	2-4
Significant difference	no		no		**yes**	
EC_{50}	11	0.3	5	2.3	9	6
Confidence interval	7-15	0-1	3-6	2-3	8-10	5-7
Significant difference	yes		**yes**		**yes**	
Growth - Dry Mass						
EC_{20}	10	0.2	14	2	6	3
Confidence interval	4-16	0.1-0.3	10-19	1-3	5.1-7.4	1.5-4.8
Significant difference	**yes**		**yes**		**yes**	
EC_{50}	49	0.7	20	4	11	6
Confidence interval	41-58	0.3-1	16-23	3.5-4.6	9-12	5-8
Significant difference	**yes**		**yes**		**yes**	

yes - weathering/aging process significantly increased toxicity.

Table 68. Effect of Weathering/Aging (W/A) of Amended Soil on Toxicity of Nitroaromatic Energetic Materials for Ryegrass Using Acetonitrile Extraction

	Fresh TNB	W/A TNB	Fresh 2,4-DNT	W/A 2,4-DNT	Fresh 2,6-DNT	W/A 2,6-DNT
Growth - Fresh Mass						
EC_{20}	45	46	11	5	18	24
Confidence interval	35-56	13-78	10-12	4-7	4-32	21-27
Significant difference	no		**yes**		no	
EC_{50}	75	83	13	7	39	39
Confidence interval	59.2-91.1	61-104	12-15	6-8	19-59	36-41
Significant difference	no		**yes**		no	
Growth - Dry Mass						
EC_{20}	56	51	11	2	26	21
Confidence interval	43-67	30-72	10-12	0-4.5	21-32	18-23
Significant difference	no		**yes**		no	
EC_{50}	89	86	13	8	39	34
Confidence interval	70-109	74-99	12-15	---	31-46	32-36
Significant difference	no				no	

yes - weathering/aging process significantly increased toxicity.

Table 69. Effect of Weathering/Aging (W/A) of Amended Soil on Toxicity of Nitroaromatic Energetic Materials for Ryegrass Using ATCLP Extraction

	Fresh TNB	W/A TNB	Fresh 2,4-DNT	W/A 2,4-DNT	Fresh 2,6-DNT	W/A 2,6-DNT
Growth - Fresh Mass						
EC_{20}	20	33	5	1.8	20	14
Confidence interval	9-31	20-45	4.5-5.5	1.3-2.4	11-28	12-16
Significant difference	no		**yes**		no	
EC_{50}	46	40	6	2.4	29	24
Confidence interval	30-62	34-46	5.6-7.1	2.1-2.7	8-49	22-26
Significant difference	no		**yes**		no	
Growth - Dry Mass						
EC_{20}	27	21	5	>1.3	20	12
Confidence interval	19-35	10-33	4.1-5.3		15-24	11-14
Significant difference	no				**yes**	
EC_{50}	49	43	6	>1.3	28	21
Confidence interval	36-62	35-51	5-7		22-34	19-22
Significant difference	no				no	

yes - weathering/aging process significantly increased toxicity.

3.6.1 Phytotoxicity of RDX and HMX.

Results of the limit tests performed with freshly amended and weathered/aged RDX or HMX amended soils (Table 50) confirmed that these two EMs were not toxic to alfalfa, Japanese millet, and ryegrass. For freshly amended and weathered/aged RDX amended soils, RDX was not toxic at concentrations of 9740 and 9537 mg kg^{-1}, respectively. For freshly amended and weathered/aged HMX amended soils (unbounded NOEC), HMX was not toxic at concentrations of 10410 and 9340 mg kg^{-1}, respectively. Furthermore, significant ($p < 0.0001$) growth stimulation was observed in Japanese millet and ryegrass exposed to these high concentrations of RDX or HMX (Tables 55 through 62).

3.6.2 Phytotoxicity of TNB.

3.6.2.1 Freshly Amended Soils.

The TNB affected germination of all plant species tested within the concentration ranges selected for definitive test (Table 44). Based on acetonitrile extractable concentrations of TNB, the bounded NOEC and LOEC values for Japanese millet were 64 and 125 mg kg^{-1}, respectively (Table 55). Because hormetic responses were measured at low TNB concentrations for alfalfa and ryegrass germination, the bounded NOAEC and LOAEC values, based on acetonitrile extractable concentrations, were 88 and 171 and 39 and 125 mg kg^{-1}, respectively (Tables 51 and 59). Based on ATCLP extractable concentrations, the bounded NOEC and LOEC values were 34 and 71 for Japanese millet (Table 56). The bounded NOAEC and LOAEC values based on ATCLP extractable concentrations were 53 and 123 for alfalfa and 18 and 71 mg kg^{-1} for ryegrass (Tables 52 and 60). The EC_{50} and EC_{20} values of TNB for germination for alfalfa, Japanese millet, and ryegrass, based on acetonitrile extractable concentrations, were 172 and 145; 204 and 109; and 95 and 28 mg kg^{-1}, respectively; and 123 and 30; 168 and 63; and 49 and 32 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

For the growth endpoint using fresh shoot mass, the NOEC/NOAEC and LOEC/LOAEC values based on acetonitrile extractable concentrations of TNB for alfalfa (unbounded NOEC), Japanese millet (bounded NOAEC), and ryegrass (bounded NOAEC) were 5 and 39, 8 and 22, and 39 and 125 mg kg^{-1}, respectively; and 0.6 and 18, 1.5 and 6, and 18 and 71 mg kg^{-1}, respectively; using ATCLP extractable concentrations. Using dry shoot mass, the bounded NOEC/NOAEC and LOEC/LOAEC values, based on acetonitrile extractable concentrations of TNB for alfalfa (bounded NOEC), Japanese millet (bounded NOAEC), and ryegrass (bounded NOEC) were 39 and 88, 22 and 64, and 39 and 125 (bounded NOAEC) mg kg^{-1}, respectively; and 18 and 53, 6 and 34, and 18 and 71 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

Concentration-response relationships for plant growth determined by nonlinear regressions are shown in Figures 1, 2, and 3. In tests with alfalfa, the logistic Gompertz model had the best fit for data. The logistic hormetic model had the best fit for Japanese millet and ryegrass data. The EC_{50} and EC_{20} values of TNB for growth using fresh shoot mass, based on

acetonitrile extractable concentrations of TNB, were 107 and 38, 36 and 16, and 75 and 45 mg kg^{-1}, respectively for alfalfa, Japanese millet, and ryegrass; and 68 and 18, 11 and 3, and 46 and 20 mg kg^{-1}, respectively, using ATCLP extractable concentrations. For dry shoot mass, the acetonitrile extractable concentration of TNB based on EC_{50} and EC_{20} values for these species were 129 and 62, 89 and 43, and 89 and 56 mg kg^{-1}, respectively; and 86 and 34, 49 and 10, and 49 and 27 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

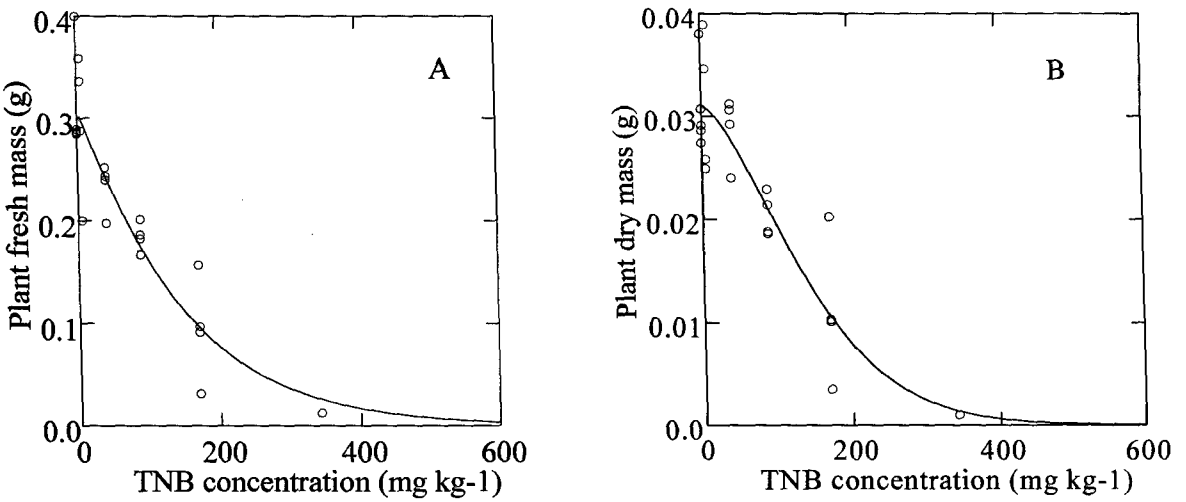

Figure 1. Effect of Freshly Amended TNB (Acetonitrile Extraction) on Alfalfa Shoot Growth (Fresh [A] and Dry [B] Mass)

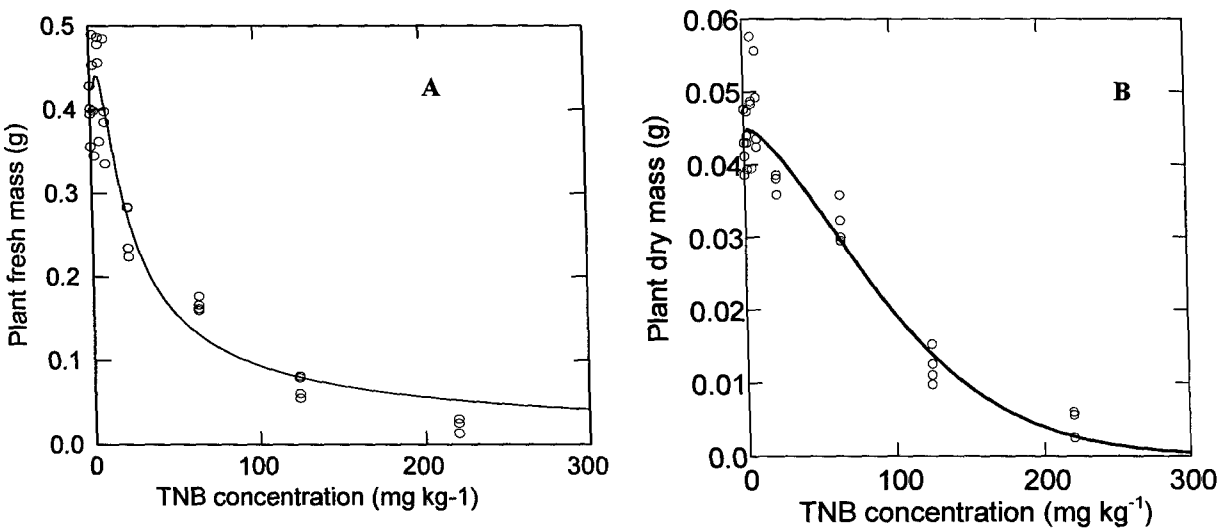

Figure 2. Effect of Freshly Amended TNB (Acetonitrile Extraction) on Japanese Millet Shoot Growth (Fresh [A] and Dry [B] Mass)

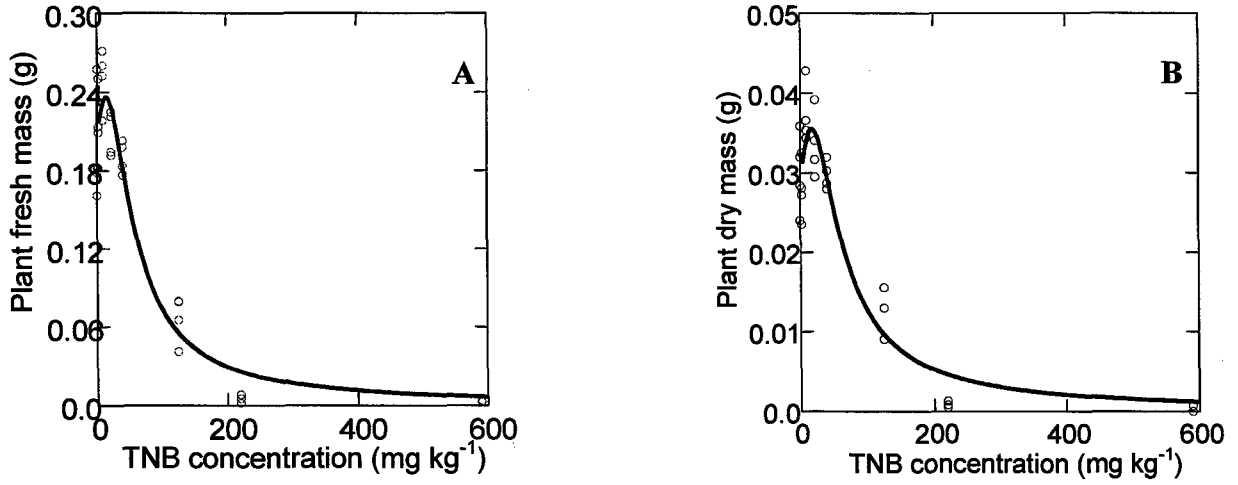

Figure 3. Effect of Freshly Amended TNB (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

3.6.2.2 Weathered/Aged Amended Soils.

Based on 95% confidence intervals, weathering/aging of amended soils increased the TNB toxicity to Japanese millet. For germination, the bounded NOEC and LOEC values, based on acetonitrile extractable concentrations of TNB for Japanese millet and ryegrass, were 81 and 197 mg kg^{-1} for both plant species (Tables 57 and 61). Because hormetic response was measured at TNB low concentrations for alfalfa germination, the bounded NOAEC and LOAEC values, based on acetonitrile extractable concentrations, were 22 and 114 mg kg^{-1} (Table 53). The bounded NOEC/NOAEC and LOEC/LOAEC values, based on ATCLP extractable concentrations for alfalfa (bounded NOAEC), Japanese millet (bounded NOEC) and ryegrass (bounded NOEC), were 7 and 67, 39 and 126, and 0.5 and 39 mg kg^{-1}, respectively. The EC_{50} and EC_{20} values of TNB for germination for alfalfa, Japanese millet, and ryegrass, based on acetonitrile extractable concentrations of TNB, were 114 and 109, 163 and 139, and 150 and 107 mg kg^{-1}, respectively, and 67 and 64, 98 and 80, and 88 and 57 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

For growth using fresh shoot mass and based on acetonitrile extractable concentrations of TNB for alfalfa, Japanese millet (unbounded LOEC), and ryegrass (unbounded LOEC), the bounded NOEC and LOEC values based were 22 and 114, <0.3 and 0.3, and <0.3 and 0.3 mg kg^{-1}, respectively, and 7 and 67, <1.4 and 1.4, and 0.5 and 39 (NOAEC/LOAEC) mg kg^{-1}, respectively, using ATCLP extractable concentrations. Using dry shoot mass and based on acetonitrile extractable concentrations of TNB for alfalfa, Japanese millet (unbounded LOEC), and ryegrass, the bounded NOEC and LOEC values were 22 and 114, <0.3 and 0.3, and 2 and 81 mg kg^{-1}, respectively, and 7 and 67, <1.4 and 1.4, and 0.5 and 39 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

Concentration-response relationships for plant growth determined by nonlinear regressions are shown in Figures 4, 5, and 6. The nonlinear regression model selection based on the fit for the data was species and endpoint specific in the weathered/aged TNB amended soils. The logistic Gompertz model had the best fit for data in tests with alfalfa and ryegrass. The exponential model had the best fit for Japanese millet data. Using fresh shoot mass based on acetonitrile extractable concentrations of TNB, the EC_{50} and EC_{20} values for alfalfa, Japanese millet, and ryegrass growth were 63 and 20, 0.9 and 0.3, and 83 and 46 mg kg^{-1}, respectively, and 29 and 7, 0.3 and 0.1, and 40 and 33 mg kg^{-1}, respectively, using ATCLP extractable concentrations. For dry shoot mass, the acetonitrile extractable concentration based EC_{50} and EC_{20} values of TNB for these species were 92 and 46, 2 and 0.7, and 86 and 51 mg kg^{-1}, respectively, and 51 and 22, 0.7 and 0.2, and 43 and 21 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

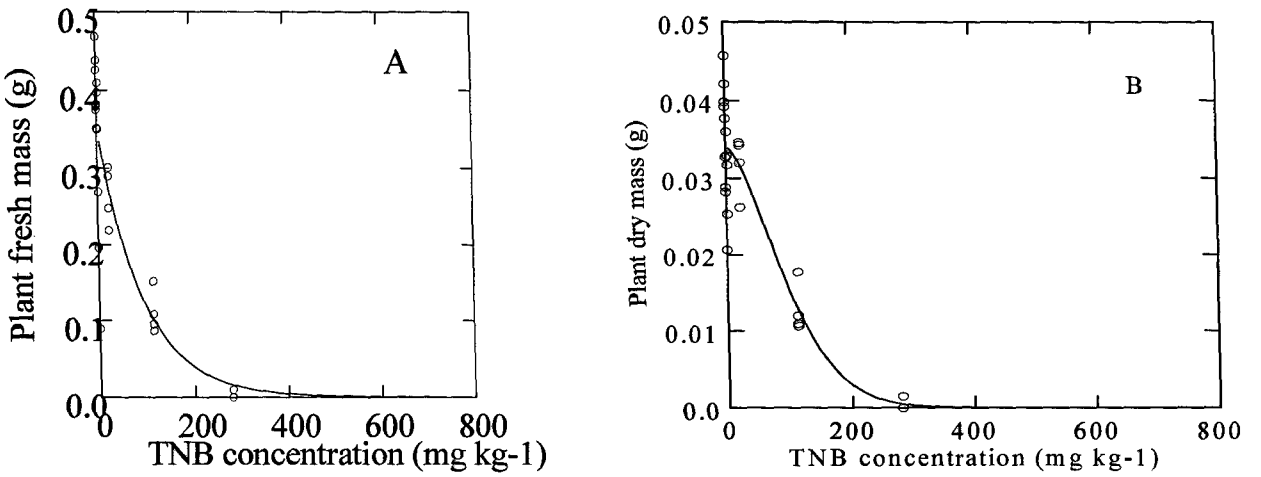

Figure 4. Effect of Weathered/Aged TNB (Acetonitrile Extraction) on Alfalfa Shoot Growth (Fresh [A] and Dry [B] Mass)

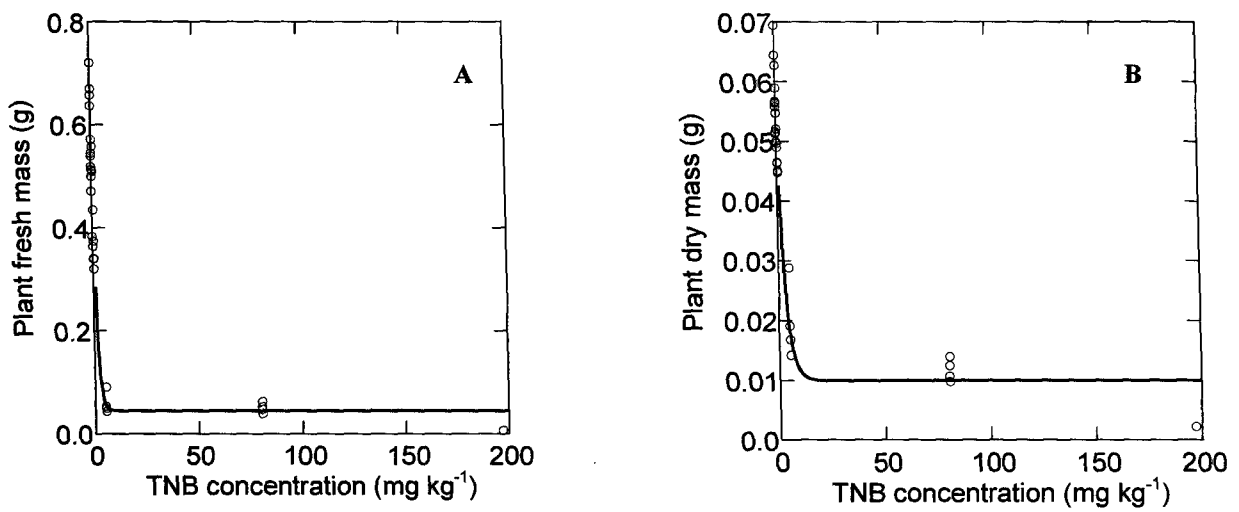

Figure 5. Effect of Freshly Amended TNB (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

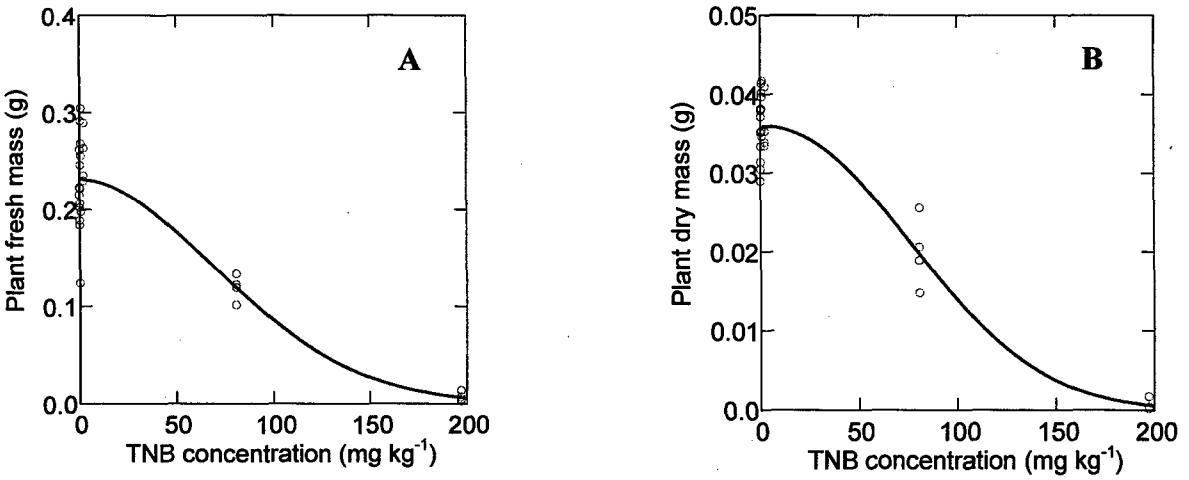

Figure 6. Effect of Weathered/Aged TNB (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

3.6.3 Phytotoxicity of 2,4-DNT.

3.6.3.1 Freshly Amended Soils.

Germination of all plant species tested was affected by 2,4-DNT within the concentration ranges selected for definitive test (Table 46). The bounded NOEC and LOEC values, based on acetonitrile extractable concentrations of 2,4-DNT for alfalfa and Japanese millet, were 47 and 99, and 9 and 22, respectively (Tables 51 and 55). Because hormetic response was elicted at low 2,4-DNT concentrations for ryegrass germination, the bounded NOAEC and LOAEC values, based on acetonitrile extractable concentrations of 2,4-DNT, were 9 and 17 mg kg^{-1}, respectively (Table 59). The NOEC/NOAEC and LOEC/LOAEC values, based on ATCLP extractable concentrations, were 27 and 69, 4 and 12, and 4 and 9 mg kg^{-1}, respectively (Tables 52, 56, and 60). The EC_{50} and EC_{20} values for germination for alfalfa, Japanese millet, and ryegrass based on acetonitrile extractable concentrations of 2,4-DNT were >47, 70, and 55, and 16 and 8 mg kg^{-1}, respectively, and >39 and 39, 45 and 33, and 8 and 3 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

Hormetic response was measured for ryegrass growth at low concentrations of freshly amended 2,4-DNT. For growth using fresh shoot mass, the NOEC/NOAEC and LOEC/LOAEC values, based on acetonitrile extractable concentrations of 2,4-DNT for alfalfa (unbounded LOEC), Japanese millet (bounded NOEC), and ryegrass (bounded NOAEC), were <4.7 and 4.7, 1.0 and 4.7, and 2 and 4 mg kg^{-1}, respectively (Tables 51, 55, and 59). The NOEC and LOEC values, based on ATCLP extractable concentrations for alfalfa (unbounded LOEC), Japanese millet (unbounded LOEC), and ryegrass (unbounded NOEC) were <1.8 and 1.8, <0.3 and 0.3, and 0.3 and 0.8 mg kg^{-1}, respectively (Tables 52, 56, and 60). Using dry shoot mass, the NOEC/NOAEC and LOEC/LOAEC values based on acetonitrile extractable concentrations of 2,4-DNT for alfalfa (unbounded LOEC), Japanese millet (bounded NOEC), and ryegrass (bounded NOAEC) were <5 and 5, 5 and 9, and 9 and 17 mg kg^{-1}, respectively, and <1.8 and 1.8, 1.8 and 4, and 4 and 9 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

Concentration-response relationships for plant growth determined by nonlinear regressions are shown in Figures 7, 8, and 9. The logistic Gompertz model had the best fit for data in tests with alfalfa and Japanese millet. The logistic hormetic model had the best fit for ryegrass data. The EC_{50} and EC_{20} values of 2,4-DNT for growth using fresh shoot mass based on acetonitrile extractable concentrations were 38 and 11, 10 and 4, and 13 and 11 mg kg^{-1}, respectively, and 27 and 10, 5 and 1, and 6 and 5 mg kg^{-1}, respectively, using ATCLP extractable concentrations. For dry shoot mass, the acetonitrile extractable concentration based EC_{50} and EC_{20} values of 2,4-DNT for these species were 56 and 34, 34 and 25, and 13 and 11 mg kg^{-1}, respectively, and 34 and 19, 20 and 14, and 6 and 5 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

3.6.3.2 Weathered/Aged Amended Soils.

Weathering/aging of amended soils increased the 2,4-DNT toxicity to Japanese millet and ryegrass based on 95% confidence intervals. Hormetic responses were measured at low concentrations of 2,4-DNT for alfalfa and Japanese millet germination. The bounded NOEC/NOAEC and LOEC/LOAEC values based on acetonitrile extractable concentrations of 2,4-DNT for alfalfa (NOAEC/LOAEC), Japanese millet (NOAEC/LOAEC), and ryegrass (NOEC/LOEC) were 89 and 121, 32 and 90, and 4 and 8 mg kg^{-1}, respectively. The bounded NOEC/NOAEC and LOEC/LOAEC values, based on ATCLP extractable concentrations, were 48 and 71 (NOAEC/LOAEC), 17 and 50 (NOAEC/LOAEC), and 1 and 3 (NOEC/LOEC) mg kg^{-1}, respectively. The EC_{50} and EC_{20} values of 2,4-DNT for germination for alfalfa, Japanese millet and ryegrass, based on acetonitrile extractable concentrations, were 115 and 104, >86 and 32, and >8 and >8 mg kg^{-1}, respectively, and 66 and 36, >17 and >17, and >3 and 3 mg kg^{-1}, respectively, using ATCLP extractable concentrations.

For growth using fresh shoot mass, the bounded NOEC and LOEC values based on acetonitrile extractable concentrations of 2,4-DNT for alfalfa, Japanese millet (unbounded NOEC), and ryegrass were 6 and 10, 1.3 and 4, and 4 and 8 mg kg^{-1}, respectively, and 2 and 4, 0.3 and 1.3, and 1 and 3 mg kg^{-1}, respectively, using ATCLP extractable concentrations. Hormetic responses were measured for alfalfa and ryegrass dry shoot mass. The bounded NOEC/NOAEC and LOEC/LOAEC values based on acetonitrile extractable concentrations of 2,4-DNT for alfalfa (NOAEC/LOAEC), Japanese millet (NOEC/LOEC), and ryegrass (NOAEC/LOAEC) were 6 and 10, 4 and 8, and 4 and 8 mg kg^{-1}, respectively, and 2 and 4, 1 and 3, and 1 and 3 mg kg^{-1}, respectively using ATCLP extractable concentrations.

Concentration-response relationships for plant growth determined by nonlinear regressions are shown in Figures 10, 11, and 12. The nonlinear regression model selection, based on the fit for the data, was species and endpoint specific in weathered/aged 2,4-DNT amended soils. Gompertz model had the best fit for fresh shoot mass data in tests with alfalfa, Japanese millet, and ryegrass. Logistic hormetic model had the best fit for dry shoot mass data in tests with alfalfa and ryegrass. The EC_{50} and EC_{20} values of 2,4-DNT for alfalfa, Japanese millet, and ryegrass growth, using fresh shoot mass based on acetonitrile extractable concentrations of 2,4-DNT, were 30 and 7, 6.5 and 3.5, and 7 and 5 mg kg^{-1}, respectively, and 14 and 2, 2.3 and 1.2, and 2.4 and 1.8 mg kg^{-1}, respectively, using ATCLP extractable concentrations. For dry shoot mass, the acetonitrile extractable concentration based EC_{50} and EC_{20} values of 2,4-DNT for these species were 42 and 15, 10 and 6, and 8 and 2 mg kg^{-1},

respectively, and 20 and 6, 4 and 2, and >1.3 and >1.3 mg kg^{-1}, respectively using ATCLP extractable concentrations.

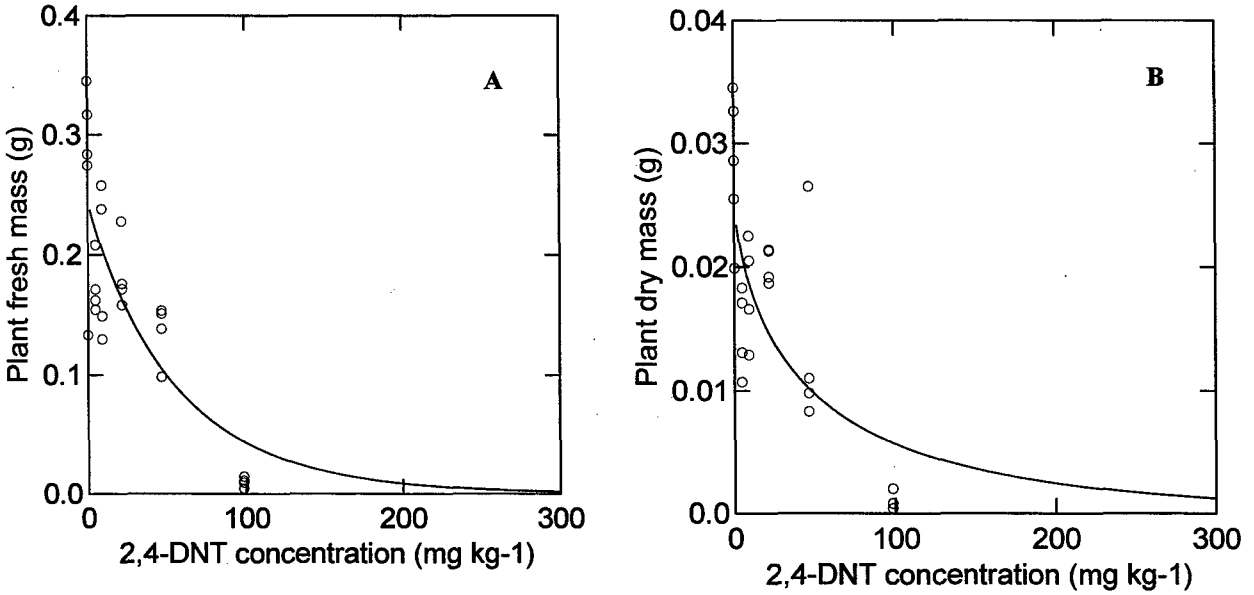

Figure 7. Effect of Freshly Amended 2,4-DNT (Acetonitrile Extraction) on Alfalfa Shoot Growth (Fresh [A] and Dry [B] Mass)

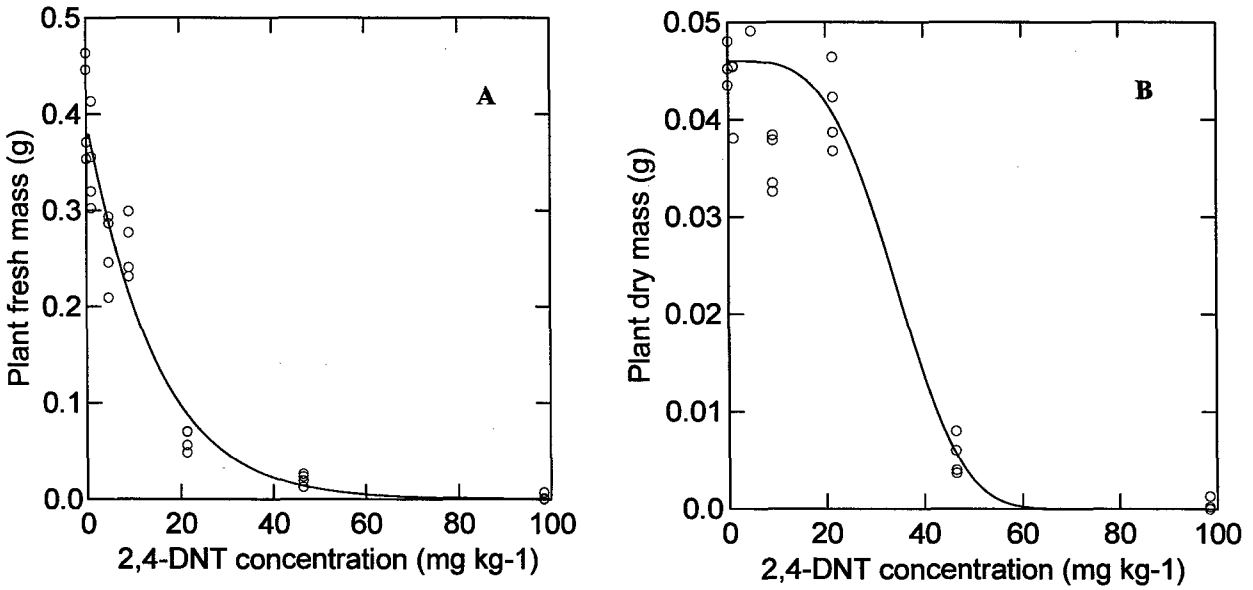

Figure 8. Effect of Freshly Amended 2,4-DNT (Acetonitrile Extraction) on Japanese Millet Shoot Growth (Fresh [A] and Dry [B] Mass)

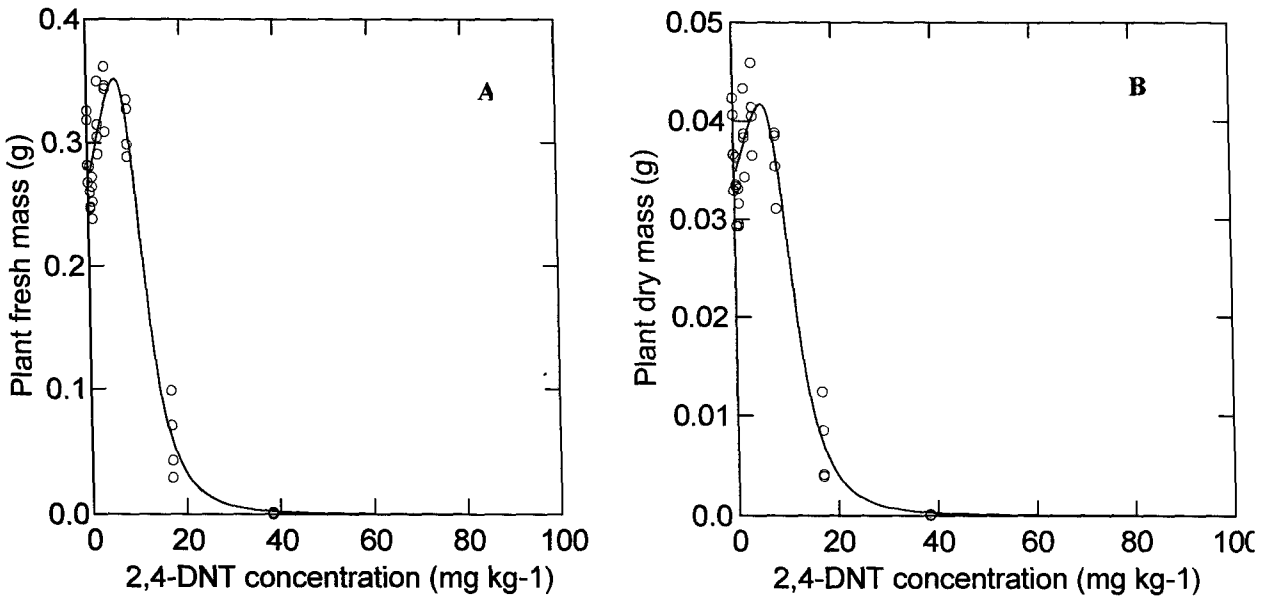

Figure 9. Effect of Freshly Amended 2,4-DNT (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

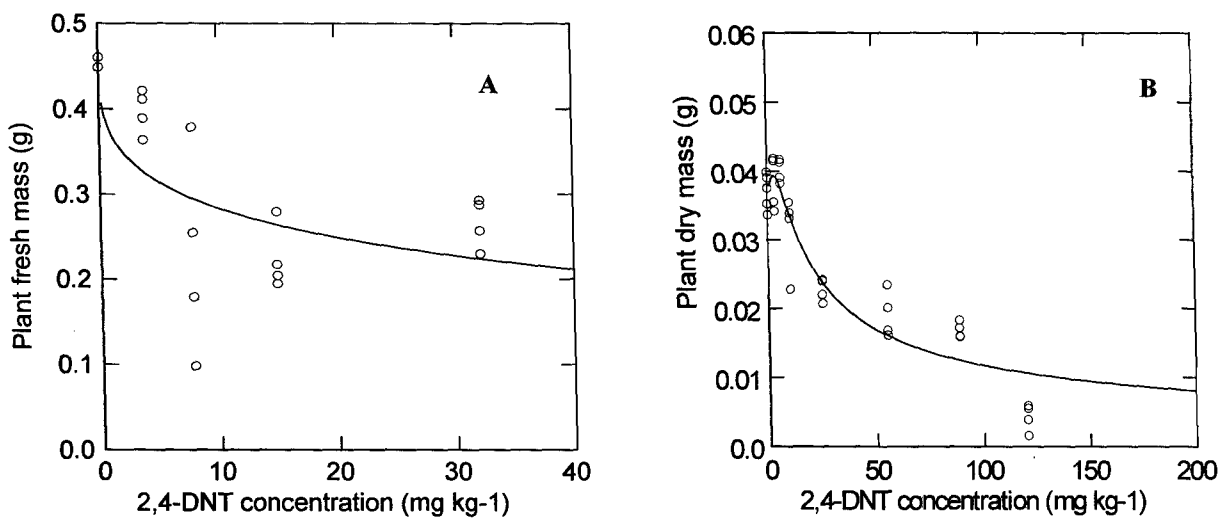

Figure 10. Effect of Weathered/Aged 2,4-DNT (Acetonitrile Extraction) on Alfalfa Shoot Growth (Fresh [A] and Dry [B] Mass)

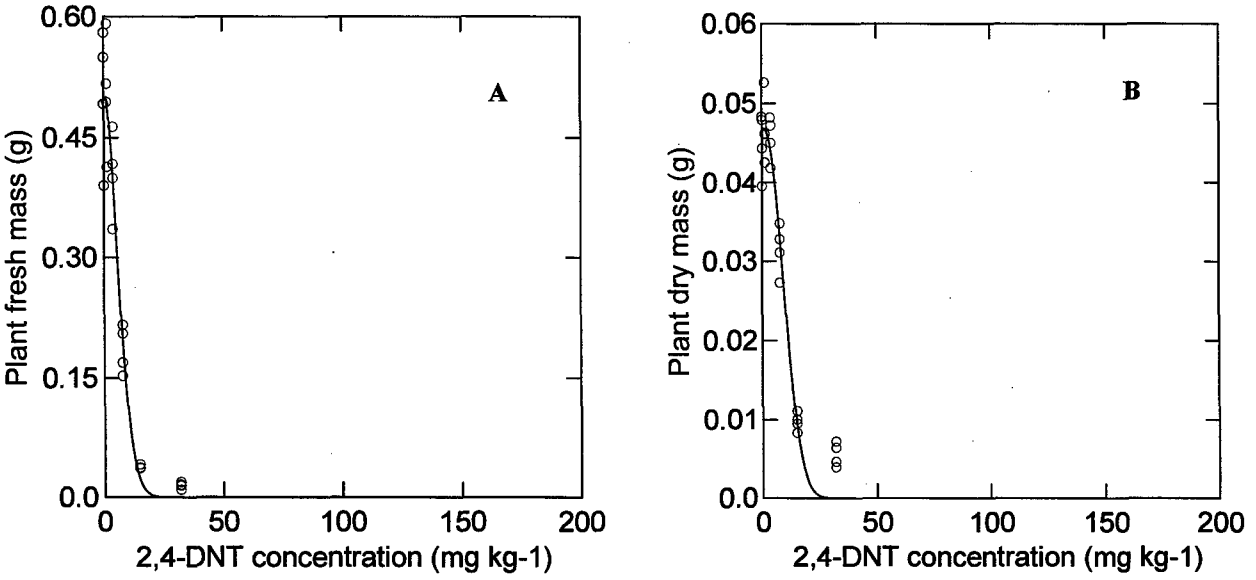

Figure 11. Effect of Weathered/Aged 2,4-DNT (Acetonitrile Extraction) on Japanese Millet Shoot Growth (Fresh [A] and Dry [B] Mass)

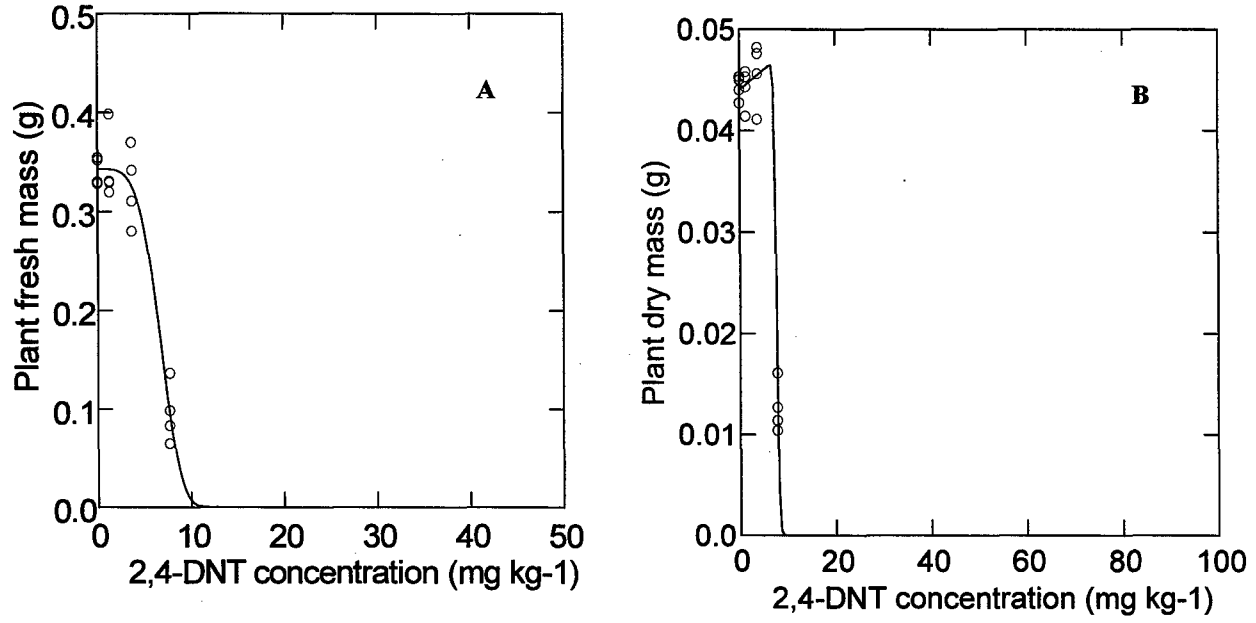

Figure 12. Effect of Weathered/Aged 2,4-DNT (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

3.6.4 Phytotoxicity of 2,6-DNT.

3.6.4.1 Freshly Amended Soils.

Germination of all plant species tested was affected by exposure to 2,6-DNT within the concentration ranges of 2,6-DNT selected for definitive tests (Table 48). The bounded NOEC and LOEC values based on acetonitrile extractable concentrations of 2,6-DNT for alfalfa, Japanese millet, and ryegrass (unbounded LOEC) were 8 and 14, 30 and 89, and <4 and 4 mg kg^{-1}, respectively (Tables 51, 55, and 59). The bounded NOEC and LOEC values based on ATCLP extractable concentrations were 4 and 8, 22 and 66, and <3 and 3 mg kg^{-1}, respectively (Tables 52, 56, and 60). The EC_{50} and EC_{20} values of 2,6-DNT for germination for alfalfa, Japanese millet and ryegrass based on acetonitrile extractable concentrations were 19 and 11, 57 and 40, and 38 and 29 mg kg^{-1}, respectively, and 12 and 6, 43 and 30, and 28 and 21 mg kg^{-1}, respectively using ATCLP extractable concentrations.

Hormetic responses were measured at low concentrations of freshly amended 2,6-DNT for Japanese millet and ryegrass growth. For growth using fresh shoot mass, the bounded NOEC/NOAEC and LOEC/LOAEC values based on acetonitrile extractable concentrations of 2,6-DNT for alfalfa (bounded NOEC), Japanese millet (unbounded NOEC) and ryegrass (bounded NOAEC) were 1.4 and 4, <4 and 4, and 30 and 89 mg kg^{-1}, respectively (Tables 51, 55, and 59), and 0.7 and 2.5, <3 and 3, and 22 and 66 mg kg^{-1}, respectively using ATCLP extractable concentrations (Tables 52, 56, and 60). Using dry shoot mass, the bounded NOEC/NOAEC and LOEC/LOAEC values based on acetonitrile extractable concentrations of 2,6-DNT for alfalfa (bounded NOEC), Japanese millet (bounded NOAEC), and ryegrass (bounded NOAEC) were 1.4 and 4, 8 and 14, and 14 and 30 mg kg^{-1}, respectively, and 0.7 and 2.5, 4 and 8, and 8 and 22 mg kg^{-1}, respectively using ATCLP extractable concentrations.

Plant growth concentration-response relationships determined by nonlinear regressions are shown in Figures 13 - 15. The logistic Gompertz model had the best fit for data in tests with alfalfa. The logistic hormetic model had the best fit for Japanese millet and ryegrass data. Using fresh shoot mass based on acetonitrile extractable concentrations, the EC_{50} and EC_{20} values of 2,6-DNT for alfalfa, Japanese millet, and ryegrass growth were 5 and 1.3, 16 and 13, and 39 and 18 mg kg^{-1}, respectively, and 3 and 0.7, 9 and 7, and 29 and 20 mg kg^{-1}, respectively. Using ATCLP extractable concentrations. For dry shoot mass, the acetonitrile extractable concentration, based EC_{50} and EC_{20} values of 2,6-DNT for these species, were 9.5 and 2.8, 18 and 11, and 39 and 26 mg kg^{-1}, respectively, and 5 and 1, 11 and 6, and 28 and 20 mg kg^{-1}, respectively using ATCLP extractable concentrations.

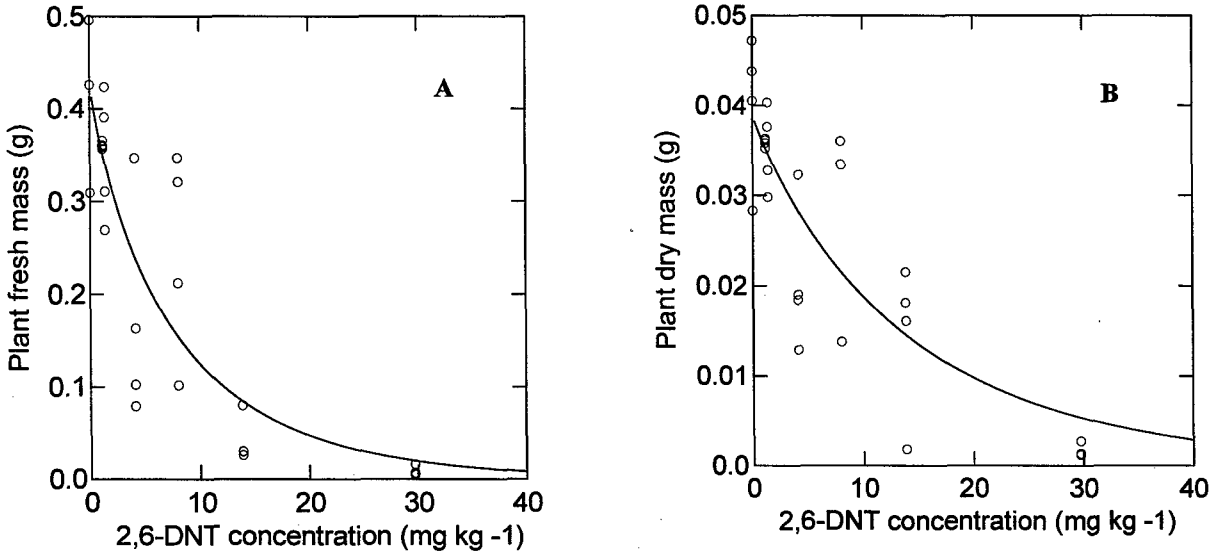

Figure 13. Effect of Freshly Amended 2,6-DNT (Acetonitrile Extraction) on Alfalfa Shoot Growth (Fresh [A] and Dry [B] Mass)

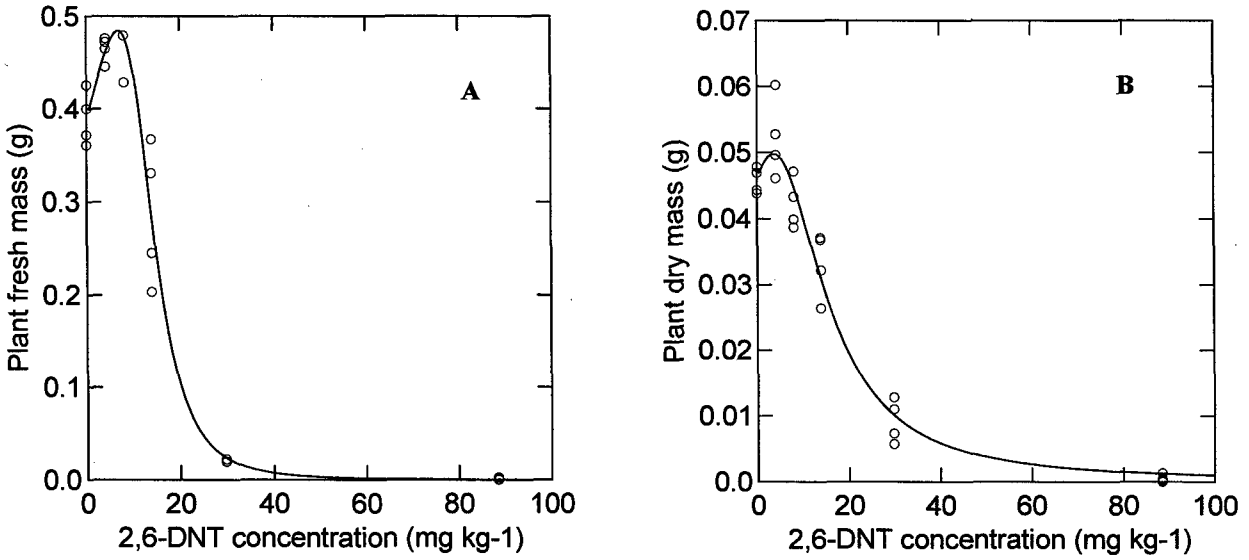

Figure 14. Effect of Freshly Amended 2,6-DNT (Acetonitrile Extraction) on Japanese Millet Shoot Growth (Fresh [A] and Dry [B] Mass)

3.6.4.2 Weathered/Aged Amended Soils.

Based on 95% confidence intervals, weathering/aging of amended soils increased the 2,6-DNT toxicity to Japanese millet. Hormetic responses were measured at low concentrations of 2,6-DNT for alfalfa and Japanese millet germination. The bounded NOEC/NOAEC and LOEC/LOAEC values, based on acetonitrile extractable concentrations of 2,6-DNT for alfalfa (NOAEC/LOAEC), Japanese millet (NOAEC/LOAEC), and ryegrass (NOEC/LOEC), were 5 and 15, 15 and 140, and 20 and 37 mg kg^{-1}, respectively. The bounded NOEC/NOAEC and LOEC/LOAEC values based on ATCLP extractable concentrations of 2,6-DNT were 3 and 10, 10 and 104, and 12 and 23 mg kg^{-1}, respectively. The EC_{50} and EC_{20} values of 2,6-DNT for germination for alfalfa, Japanese millet, and ryegrass, based on acetonitrile extractable concentrations, were 55 and 26, >15 and >15, and 54 and 42 mg kg^{-1}, respectively, and 41 and 4, 53 and 3, and 34 and 25 mg kg^{-1}, respectively using ATCLP extractable concentrations.

For growth using fresh shoot mass, the bounded NOEC and LOEC values based on acetonitrile extractable concentrations of 2,6-DNT for alfalfa, Japanese millet (unbounded NOEC for ATCLP), and ryegrass (unbounded NOEC for acetonitrile and ATCLP) were 3 and 5, 1 and 3, and 8 and 20 mg kg^{-1}, respectively, and 2 and 3, 0.6 and 1.5, and 4 and 12 mg kg^{-1}, respectively using ATCLP extractable concentrations. Using dry shoot mass, the bounded NOEC and LOEC values based on acetonitrile extractable concentrations of 2,6-DNT for alfalfa, Japanese millet and ryegrass (unbounded NOEC) were 3 and 5, 3 and 5, and 8 and 20 mg kg^{-1}, respectively, and 1 and 3, 1 and 3, and 4 and 12 mg kg^{-1}, respectively using ATCLP extractable concentrations.

Concentration-response relationships for plant growth determined by nonlinear regressions are shown in Figures 16 - 18. The nonlinear regression model selection, based on the fit for the data, was and endpoint specific in weathered/aged 2,6-DNT amended soils. The logistic hormetic model had the best fit for germination data, and the Gompertz model had the best fit for growth endpoints. The EC_{50} and EC_{20} values of 2,6-DNT for alfalfa, Japanese millet, and ryegrass growth, using fresh shoot mass based on acetonitrile extractable concentrations, were 7 and 1.6, 9 and 4.8, and 39 and 24 mg kg^{-1}, respectively, and 4 and 0.7, 6 and 3, and 24 and 14 mg kg^{-1}, respectively using ATCLP extractable concentrations. For dry shoot mass, the acetonitrile extractable concentration based EC_{50} and EC_{20} values of 2,6-DNT for these species were 5 and 0.4, 11 and 6, and 34 and 21 mg kg^{-1}, respectively, and 2 and 0.1, 6 and 3, and 21 and 12 mg kg^{-1}, respectively using ATCLP extractable concentrations.

3.6.5 Relationship Between Chemical Extraction Method and Phytotoxicity.

Coefficients of determinations (R^2) for acetonitrile and ATCLP based extractions determined in nonlinear regression analyses of the plant germination and growth data from studies with fresh and weathered/aged amended soils were compared to determine which chemical measure of exposure better correlated with toxicity (Table 63). These comparisons showed that neither extraction method had an advantage for characterizing bioavailability of EMs to the three terrestrial plant species tested in this study. This was true for freshly amended and weathered/aged amended soils.

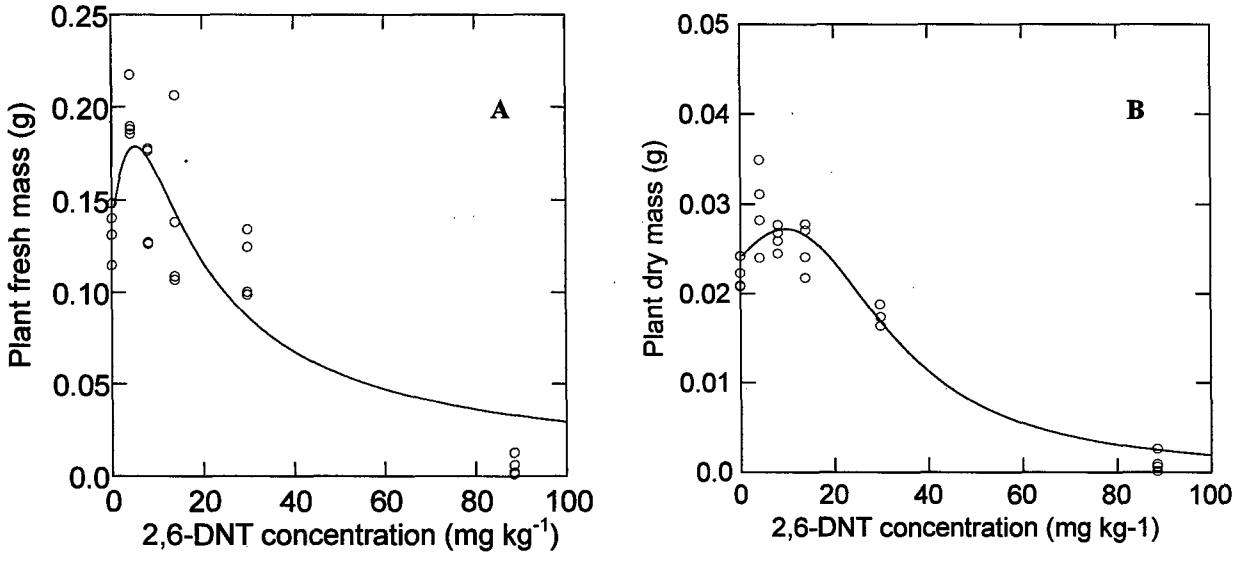

Figure 15. Effect of Freshly Amended 2,6-DNT (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

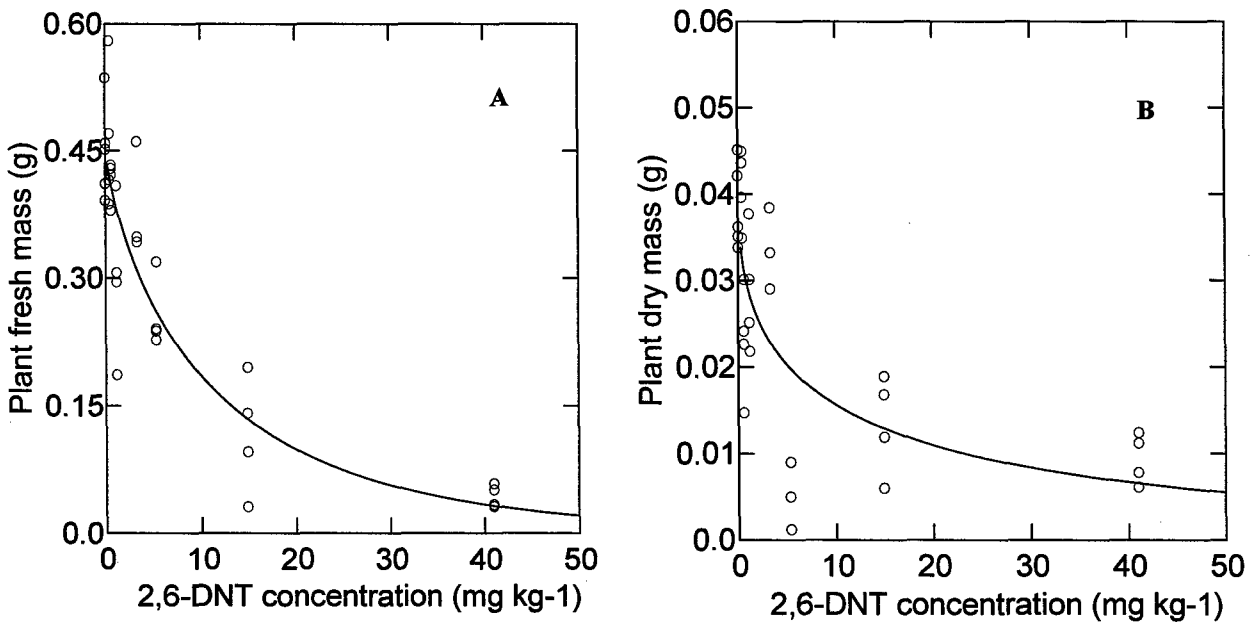

Figure 16. Effect of Weathered/Aged 2,6-DNT (Acetonitrile Extraction) on Alfalfa Shoot Growth (Fresh [A] and Dry [B] Mass)

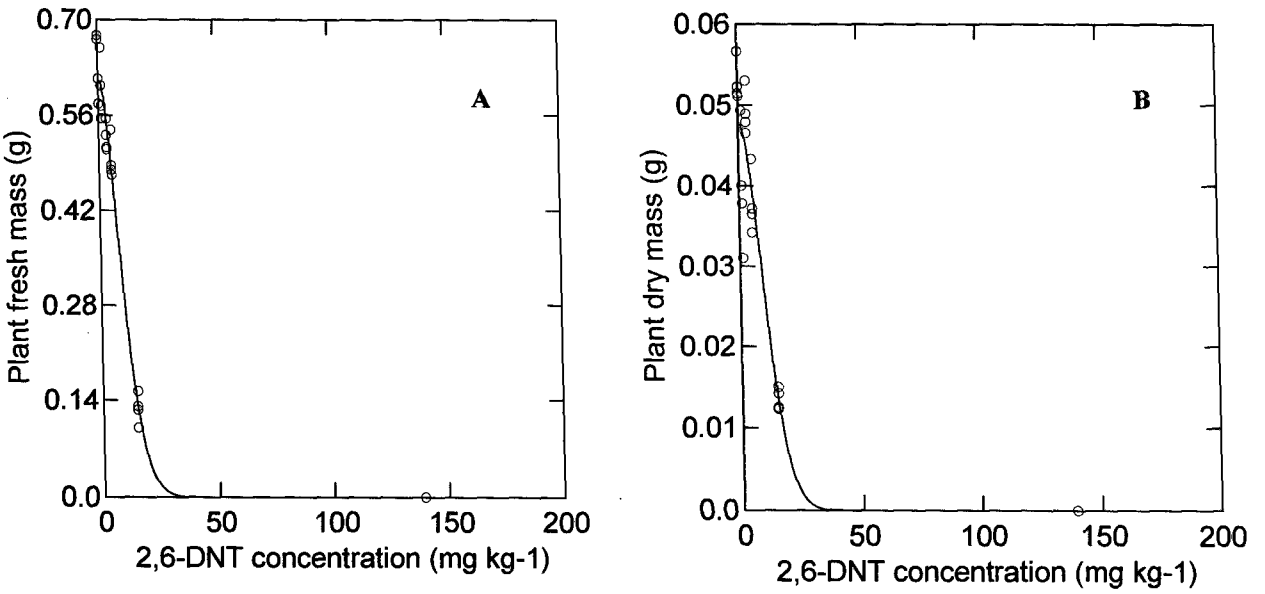

Figure 17. Effect of Weathered/Aged 2,6-DNT (Acetonitrile Extraction) on Japanese Millet Shoot Growth (Fresh [A] and Dry [B] Mass)

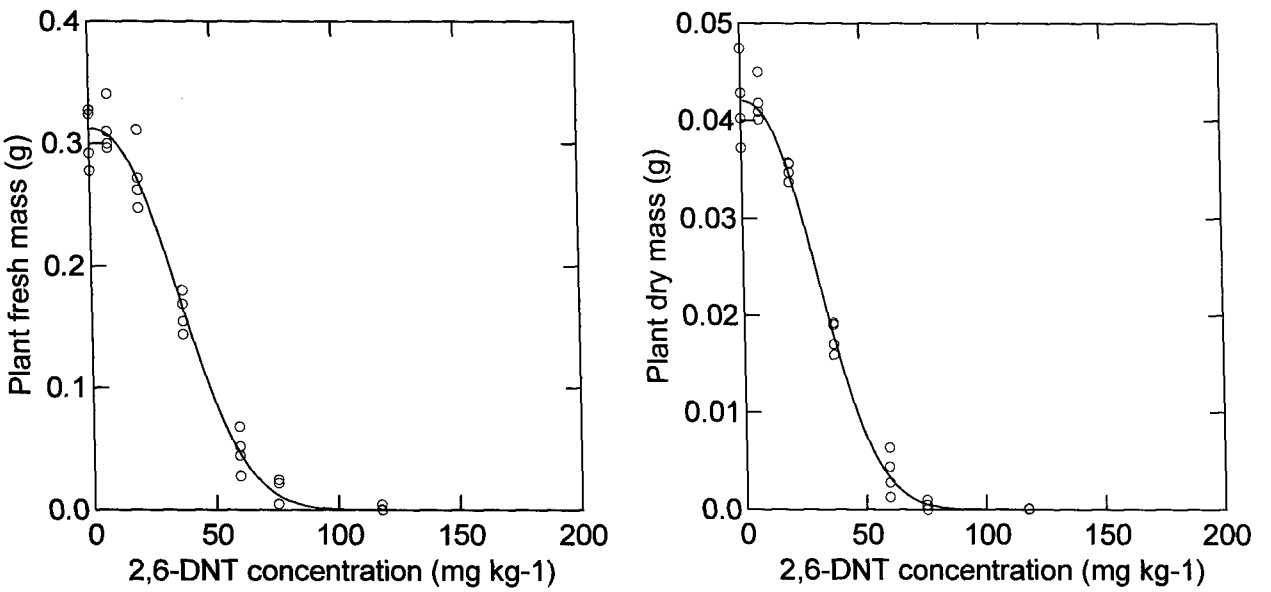

Figure 18. Effect of Weathered/Aged 2,6-DNT (Acetonitrile Extraction) on Ryegrass Shoot Growth (Fresh [A] and Dry [B] Mass)

4. DISCUSSION

Development of ecotoxicological benchmarks for energetic soil contaminants has become a critical need in recent years. These benchmarks are required for derivation of ecological soil screening levels (Eco-SSLs) for use in Ecological Risk Assessment (ERA) of contaminated sites (United States Environmental Protection Agency, 2000). The Eco-SSLs represent concentrations of chemicals in soil that, when not exceeded, will be theoretically protective of terrestrial ecosystems within specific soil boundary conditions from unacceptable harmful effects. An extensive review of literature determined that there was insufficient information for energetic material contaminants in soil to generate Eco-SSL values for terrestrial plants (United States Environmental Protection Agency, 2000). The majority of soil toxicity tests that were reported in literature used standard artificial soil with high organic matter content (10%). In contrast, our toxicity studies designed to specifically fill this knowledge gap, used a natural soil that met the criteria for Eco-SSL development because it had characteristics supporting relatively high bioavailability of energetic materials (Ems). In addition, our weathering/aging procedure applied to soils loaded with range of EM concentrations allowed us to more realistically assess the toxicity under conditions more closely resembling the potential toxic effects of RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB to terrestrial plants in the field.

4.1 Determination of Energetic Materials in Soil by Chemical Analysis.

Derivation of Eco-SSL values prioritizes ecotoxicological benchmarks that are based on measured soil concentration of a chemical over those based on nominal concentrations (USEPA, 2000). In this study, the exposure concentrations of RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB in soil were analytically determined in all definitive toxicity tests. Chemical analysis used the USEPA Method 8330A based on acetonitrile extraction of EMs from soil. Results from acetonitrile extraction of freshly amended soils showed good correlation between nominal and measured concentrations for the five energetic materials. This confirmed that the soil amendment procedure used in toxicity tests was appropriate, and the USEPA Method 8330A was efficient for quantifying the amount of energetic materials in soil.

An additional procedure that measures the water extractable portion of each EM in amended soil was performed using the Adapted Toxicity Characteristic Leaching Procedure (ATCLP). This water extractable portion of each EM was perceived to measure the bioavailable fraction of chemicals in soil pore water that is potentially better correlated with toxicity as compared to acetonitrile extracted chemical measure. The ATCLP extractable concentrations of 2,4-DNT, 2,6-DNT, and TNB freshly amended in SSL soil increased proportionally with their respective concentrations. In contrast, only 2 and 0.2 % of RDX and HMX concentrations, respectively, were ATCLP extractable in soils freshly amended with 10000 mg kg^{-1} RDX or HMX. These low ATCLP-based recoveries reflected the low water solubility of both compounds, which were reported for RDX as 42 mg L^{-1} at 20 °C (Sikka *et al.*, 1980) and as 60 mg L^{-1} at 25 °C (Banerjee *et al.*, 1980). The water solubility of HMX was reported between 5 and 6.6 mg L^{-1} at 25 and 20 °C, respectively (Glover and Hoffsommer, 1973; McLellan *et al.*, 1992).

Assessment of the EM toxicities to terrestrial plants for Eco-SSL development included studies with weathered and aged EM amended soils to simulate more closely the exposure effects in the field. Weathering/aging of chemicals in soil may reduce exposure of plants to EMs due to photodecomposition, hydrolysis, reaction with organic matter, sorption, fixation, precipitation, immobilization, occlusion, microbial transformation, and other fate processes that commonly occur at contaminated sites. These fate processes can either reduce the amount of chemical that is bioavailable, compared to tests conducted with freshly amended soils, or they may reveal increased toxicity due to the presence of more toxic transformation products.

Acetonitrile extractable concentrations of TNB, 2,4-DNT, and 2,6-DNT were significantly reduced in weathered/aged amended soils. Transformation of TNB was evident in soil amended with low concentrations ranging from 2 to 80 mg kg^{-1} and was not proportional to the amount of EM in soil at higher concentrations ranging from 120 to 1600 mg kg^{-1}. A transformation product of TNB, 3,5-DNA was detected in weathered/aged amended soil, which suggested that TNB was undergoing microbial and/or photolytic degradation. Two metabolites of 2,4-DNT, including 2-A-4 NT and 4-A-2 NT 2,4-DNT, were detected in weathered/aged soil amended with low concentrations of 2,4-DNT, confirming that this EM was also undergoing transformation. Bacteria able to mineralize 2,4-DNT (e.g., *Pseudomonas sp.* strain), have been isolated from a variety of contaminated soils (Spain, 1995). The 2,4-DNT and 2,6-DNT are readily biotransformed by *Pseudomonas sp.* and eventually eliminated as nitrite (Spanggord *et al.*, 1991; Kaplan, 1992; and Haidor and Ramos, 1996). In our study, transformation of 2,4-DNT was less pronounced at higher concentrations of 600 and 1200 mg kg^{-1}. The 2,6-DNT was transformed in all concentrations tested although no measurable quantities of transformation products were detected in the weathered/aged amended soils. Data analysis of ATCLP/ acetonitrile ratios confirmed that the water extractable portions of TNB and DNTs in weathered/aged amended soils were significantly lower compared with freshly amended soils. Presumably, this was a result of fate processes in the amended soils undergoing weathering and aging. In contrast to nitroaromatic EMs, there were no appreciable reductions in RDX or HMX acetonitrile extractable concentrations after the 3-month weathering/aging period, and their water extractable fractions remained low in weathered/aged soil. Under aerobic conditions, RDX and HMX transformation is limited (Rosenblatt *et al.*, 1991; and Hawari and Halasz, 2002). Soil contaminated with RDX and bioaugmented with *Rhodococcus* bacterial strain showed a limited 10% mineralization (Jones *et al.*, 1995). Increasing the concentration of RDX gradually decreased mineralization to undetectable levels at concentrations above 3000 mg kg^{-1}, which is below the 10000 mg kg^{-1} tested in the present study. Overall, chemical analyses demonstrated that EM exposure conditions of terrestrial plants in weathered/aged amended soils differed from those of freshly amended soils. Including the weathering/aging component in the EM toxicity assessments allowed us to incorporate potential alterations in EM bioavailability at contaminated sites in the ecotoxicological benchmarks development for terrestrial plants.

The fate of EMs in soil can modify the exposure concentrations of plant species tested and affect the accuracy of ecotoxicological benchmarks determined from concentration-response relationships based on the initial chemical concentrations. Assessment of the change in chemical concentration during the exposure period is particularly important for organic compounds with high transformation rates and/or sorption ability when weathering/aging of the EMs is not carried out prior to toxicity testing. Furthermore, it has been demonstrated that TNT

(a nitroaromatic compound) has a stronger sorption ability that of HMX and RDX, both nitro-heterocyclic compounds (Monteil-Rivera *et al.*, 2003). For that reason, we measured concentrations of all EMs tested at the end of each toxicity assay in addition to analytical determinations at the beginning of the assay. Results showed that concentrations of nitroaromatic EMs were considerably decreased in soil freshly amended with low treatment concentrations, and the decrease in acetonitrile extractability of either TNB, 2,4-DNT or 2,6-DNT was inversely related to the initial (T_0) acetonitrile extractable concentrations of these EMs. At concentrations below 100 mg kg^{-1}, the decrease in concentrations of either TNB, 2,4-DNT or 2,6-DNT ranged from 43 to 100 %, while above that treatment concentration, decrease in concentrations ranged from 0 to 63 %. There was almost no RDX concentration decrease (4%) in the single 10,000 mg kg^{-1} treatment. The HMX concentration decrease (in the single 10,000 mg kg^{-1} treatment) ranged from 10 to 17 % of the initial acetonitrile extractable concentration in freshly amended soil to 0 to 4 %t in weathered/aged amended soil in tests with three plant species. Decrease in concentrations of TNB and 2,6-DNT, during the toxicity tests in weathered/aged amended soil, were similar to decreases in freshly amended soil, whereas, 2,4-DNT concentration decrease in weathered/aged soil was less than that in freshly amended soil.

Decreased concentrations of freshly amended test compounds, during toxicity testing, posed the challenge of selecting the appropriate concentrations to use for estimating the concentration-response relationship. The initial chemical concentrations were used in nonlinear regression analyses to estimate EC_{50} and EC_{20} values because it was impossible to determine what level of constantly decreasing exposure concentration could account for toxic response (or a portion of such response). This choice was based on the assumption that for weathered/aged amended soil, the initial concentration was the best representation of the exposure condition of test species and was most appropriate for Eco-SSL derivation. In future investigations, alternative approaches may either include measuring concentration over duration of test and expressing "dose" as area under the curve or using a geometric mean of chemical concentrations determined during the test. An alternative to soil analytical determination approach can be the use of organism chemical residue as measure of exposure.

The persistent concentration decrease of nitroaromatic Ems, even in weathered/aged amended soil, shows clearly the important role terrestrial plants play in the fate of these compounds in soil. Although substantial portions of these EMs were degraded/ transformed during the 3-month weathering and aging period, presence of plants further accelerated either the degradation/transformation of TNB, 2,4-DNT or 2,6-DNT from amended soil during a short period of toxicity testing. Both plant uptake and stimulation of rhizosphere processes could contribute to the decrease of test compounds, and additional studies are required to elucidate the specific mechanisms. It has been reported that RDX and HMX are bioaccumulated by some plants (French *et al.*, 2001; Pennington and Brannon, 2002). The present study results showed that capacity of plants to facilitate the degradation of nitroaromatic compounds beyond the microbially and/or abiotically mediated degradation pathways of plant-free soil was concentration dependent. At concentrations below 100 mg kg^{-1}, plants contributed to degradation of up to 70% of TNB, 30% of 2,4-DNT, and 100% of 2,6-DNT, while the compound concentration decrease was not as important at higher soil concentrations.

This clearly shows the importance of developing Eco-SSL values that are protective of the terrestrial plant communities potentially capable of contributing to degradation, detoxification, and ultimately, to the remediation of energetic nitroaromatic contaminated sites.

Coefficients of determinations (R^2) for acetonitrile and ATCLP based extractions determined in nonlinear regression analyses of the plant germination and growth data from studies with fresh and weathered/aged amended soils were compared to determine which chemical measure of exposure better correlated with toxicity. These comparisons of coefficients of determinations showed that neither extraction method had an advantage for characterizing bioavailability of EMs to the three terrestrial plant species tested in this study. This was true for freshly amended and weathered/aged amended soils. This result supports our decision to develop Eco-SSLs for explosives contaminants in soil on the basis of acetonitrile extraction of test compounds. The acetonitrile extraction-based Eco-SSL values will be especially useful for Ecological Risk Assessment at contaminated sites because EM concentrations determined during site characterization are usually based on acetonitrile extraction by the USEPA Method 8330A.

4.2 Plant Toxicity Tests In Sassafras Sandy Loam Soil.

We assessed the toxicity of two explosives RDX and HMX, and three TNT by-products 2,4-DNT, 2,6-DNT, and TNB to alfalfa, corn, lettuce, Japanese millet, and perennial ryegrass in a natural soil, Sassafras sandy loam. The SSL soil had low organic matter and clay content, low cation exchange capacity, and high sand content. Such characteristics supported relatively high bioavailability of energetic contaminants in soil, preferred for Eco-SSLs development. Preliminary range-finding tests identified the three plant species most sensitive to energetic materials tested and with performance parameters in SSL soil required by the validity criteria of standardized toxicity tests. These species included a dicotyledonous symbiotic species, alfalfa, and two monocotyledonous species (Japanese millet and ryegrass).

Nitro-heterocyclic explosives RDX or HMX did not adversely affect alfalfa, Japanese millet, or ryegrass seedling emergence or growth at concentrations of 9740 and 10411 mg kg^{-1}, respectively, in the definitive limit tests with either freshly amended or weathered/aged amended SSL soil. Significant growth stimulation was observed in studies with Japanese millet and ryegrass exposed to these concentrations of RDX or HMX. Relatively low exposure concentrations of these EMs in pore water of amended soil, resulting from their low solubility levels in water, could contribute to these results. The solubility levels in water at 20 °C of RDX and HMX are 42 and 6.6 mg L^{-1}, respectively (Sikka *et al.*, 1980; McLellan *et al.*, 1992).

Dinitrotoluenes (DNTs) and trinitrobenzene (TNB) adversely affected alfalfa, Japanese millet, and ryegrass in the definitive toxicity tests at concentration ranges selected from the range-finding tests. Plant growth was a more sensitive endpoint compared with seedling emergence in freshly amended and weathered/aged amended soils. (Sunahara *et al.*, 2001) suggested that lower sensitivity of seedling emergence based toxicity endpoint could be related to the use of energy reserves by cotyledons plants for germination. Fresh shoot mass was a more sensitive measurement endpoint compared with dry shoot mass, as evidenced by lower EC_{20} and EC_{50} values for TNB, 2,4-DNT and 2,6-DNT in most tests. These results support strongly the

USEPA decision of giving a higher priority to ecotoxicological benchmarks based on growth over other assessment endpoints (e.g., seedling emergence, root elongation) for developing Eco-SSLs for terrestrial plants (USEPA, 2000).

Definitive toxicity tests with freshly amended and weathered/aged amended soils showed that EM toxicity order, based on EC_{20} values for plant growth (fresh or dry shoot mass) in tests with alfalfa, was 2,6-DNT > 2,4-DNT > TNB. Toxicity order for these endpoints in tests with ryegrass was 2,4-DNT > 2,6-DNT > TNB. Toxicity order varied for Japanese mille, which depended on exposure type and measurement endpoint used. In freshly amended soil, toxicity order was 2,6-DNT > 2,4-DNT > TNB, based on dry mass, and 2,4-DNT > 2,6-DNT > TNB, based on fresh mass. In weathered/aged amended soils, toxicity order, based on fresh or dry mass, was TNB > 2,4-DNT ≥ 2,6-DNT. These results show that toxicity of these nitroaromatic energetic materials varied among the three test species, and the USEPA requirement of using multiple species for Eco-SSLs development (USEPA, 2000) is well justified.

Because this study was designed to produce benchmark data for development of Eco-SSLs for explosives contaminants in soil, the results of this study may not directly compare to those of other studies in the literature because none of them were designed to specifically quantify EM toxicity to terrestrial plants under Eco-SSL conditions of testing. Studies on soil-based phytotoxicity of explosives to higher plants are scant (Sunahara *et al.*, 2001). Simini *et al.* (1995) reported statistically significant reductions in cucumber and radish height and survival in soils with mixture of energetic contaminants containing up to 3574 mg kg^{-1} RDX, 3000 mg kg^{-1} HMX, 2,655 mg kg^{-1} TNT, and up to 180 mg kg^{-1} of byproducts of TNT manufacturing and/or degradation. However, these results cannot be directly compared with our studies due to compounding effects of contaminant mixtures in these studies. Robidoux *et al.* (2003) estimated IC_{20} values of 204 and 3113 mg kg^{-1} TNT for lettuce seedling emergence in forest soil and artificial soil (silica), respectively. Exposure of barley seeds to TNT in forest soil or silica produced IC_{20} values of 398, 139, 272, and < 91 mg kg^{-1} TNT for barley seedling emergence, fresh shoot mass, dry shoot mass, and root mass in forest soil, whereas these values were 8133, 8133, 133, 1199, and < 56 mg kg^{-1} TNT in artificial soil, respectively (Robidoux *et al.*, 2003). Winfield *et al.* (1999) found that exposure to RDX (up to 4000 mg kg^{-1} soil) during early life stage resulted in adverse responses in sensitive terrestrial plants such as sunflower and sanfroin. Bean, wheat, and blando brome plants were grown in soil amended with 10 mg kg^{-1} RDX (Cataldo *et al.*, 1989), and bush bean (*Phaseolus vulgaris*) was also hydroponically exposed to 10 mg L^{-1} RDX for 1 or 7 days (Harvey *et al.*, 1991), but effects on growth were not reported. Although a screening benchmark of 100 mg kg^{-1} RDX soil was determined by Talmage *et al.* (1999), confidence in the benchmark is low because the available data were insufficient. Results of our studies showing no adverse effects of RDX or HMX at 10,000 mg kg^{-1} on the terrestrial plants tested are in disagreement with these reported results.

Hormesis, a stimulatory effect caused by low levels of potentially toxic chemicals followed by inhibitory effects at higher concentrations (Stebbing, 1982; Calabrese *et al.*, 1987), was observed in all plant species exposed to TNB, 2,4-DNT, and 2,6-DNT. Hormesis has been reported in plants exposed to heavy metals and aromatic hydrocarbons (Stebbing, 1982; Calabrese *et al.*, 1987). Hormetic responses were reported in EM exposure studies for microbial

nitrogen fixation activity at TNT concentrations in soil of 200 and 400 mg kg^{-1} (Gong *et al.*, 1999). Hormetic responses have also been shown in aquatic investigations, including offspring production by *Daphnia magna* exposed to 0.08 mg L^{-1} TNT (Bailey *et al.*, 1985), egg production per female fathead minnow exposed to 6.3 mg L^{-1} RDX (Bentley *et al.*, 1977), and density of *Selanastrum capricornutum* cells, based on total chlorophyll measures following HMX exposure ranging 36-572 mg L^{-1} (Bentley *et al.*, 1984). To date, no studies have investigated the mechanisms responsible for stimulating effects of these explosives at specific concentrations. Stevens *et al.* (2002) suggested that these mechanisms could include the direct effect on test organisms through the release of metabolic products of explosives that may have a specific effect on growth and reproduction and indirect effects through increased supply of nitrogen from mineralization of explosives.

Weathering/aging of EM amended soils did not reduce the toxicity for terrestrial plant species tested. In fact, weathering/aging of 2,4-DNT, 2,6-DNT, or TNB amended soils significantly increased toxicity for Japanese millet, which was the most sensitive species among the plant species tested. Weathering/aging of amended soils also significantly increased the toxicity of 2,4-DNT for ryegrass. Specific mechanisms of changes in the toxicity of EMs in weathered/aged amended soil are unknown. Transformation products produced during the weathering and aging process may be more toxic to soil organisms compared with the parent material and can contribute to the increased toxicity in weathered/aged amended soil. Dodard *et al.* (1999) investigated the toxic effects of 2,4-DNT and 2,6-DNT, and their respective metabolites using the 15-min Microtox (*Vibrio fischeri*; marine bacteria) and 96-hr freshwater green alga (*S. capricornutum*) growth inhibition tests. The toxicities of DNTs were species-dependent. The 2,4-DNT was more toxic than 2,6-DNT to *S. capricornutum*, whereas the reverse was true in the test with *Vibrio fischeri*. The authors reported that the reduced metabolites of 2,6-DNT tested were less toxic compared to the toxicity of parent compound. However, certain partially reduced metabolites of 2,4-DNT (4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene) were more toxic than the parent compound. These results cannot be directly compared to our study because the biotic reductive degradation pathway for 2,4-DNT and 2,6-DNT in aquatic environment contrasts with metabolic processes in the aerobic conditions of vadose zone simulated in our investigations. The reducing environment can exist in intermittently water-logged soil microsites, where more toxic metabolites of dinitrotoluenes transformation can be present. The higher toxicity of these metabolites may explain the increased toxicity of nitroaromatic energetic materials in weathered/aged amended SSL soil observed in our study. Overall results of our study showed that special consideration given to the effects of weathering and aging of energetic contaminants in soil for assessing phytotoxicity was well justified. Benchmark values generated in these investigations will contribute to developing Eco-SSLs that better represent the exposure conditions of terrestrial plants at contaminated sites. Table 70 summarizes the EC_{20} values that were submitted to the Ecological Soil Screening Level (Eco-SSLs) workgroup for quality control review by the Eco-SSL task group before inclusion in the Eco-SSL database and before being used for developing Eco-SSLs for RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB for terrestrial plants.

Table 70. Summary of the Plant Growth EC_{20} Values (mg kg^{-1}) for Freshly Amended and Weathered/Aged TNB, 2,4-DNT or 2,6-DNT Amended Sassafras Sandy Loam Soil

EC_{20} for Fresh Shoot Growth (n = 4)	Freshly Amended TNB	Fresh Amended 2,4-DNT	Fresh Amended 2,6-DNT
Alfalfa	38	11	1.3
Japanese millet	16	3.5	13
Ryegrass	45	11	18
EC_{20} for Fresh Shoot Growth (n = 4)	**Weathered/Aged TNB**	**Weathered/Aged 2,4-DNT**	**Weathered/Aged 2,6-DNT**
Alfalfa	20	7	1.6
Japanese millet	0.3	3.5	4.8
Ryegrass	46	5	24
EC_{20} for Dry Shoot Growth (n = 4)	**Freshly Amended TNB**	**Fresh Amended 2,4-DNT**	**Fresh Amended 2,6-DNT**
Alfalfa	62	34	2.8
Japanese millet	43	25	11
Ryegrass	56	11	26
EC_{20} for Dry Shoot Growth (n = 4)	**Weathered/Aged TNB**	**Weathered/Aged 2,4-DNT**	**Weathered/Aged 2,6-DNT**
Alfalfa	46	15	0.4
Japanese millet	0.7	6	6
Ryegrass	51	2	21

5. CONCLUSIONS

This study has produced ecotoxicological data for terrestrial plants alfalfa, Japanese millet and ryegrass for energetic materials hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tentrazine (HMX), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), and 1,3,5-trinitrobenzene (TNB). All ecotoxicological parameters were determined using measured chemical concentrations. This complies with U.S. Environmental Protection Agency (USEPA) preference for derivation of the ecological soil screening level (Eco-SSL) values on the basis of measured soil concentration of a chemical over those based on nominal concentrations (USEPA, 2000). Chemical analyses of freshly amended soils, using the USEPA Method 8330A, showed good correlation between nominal and measured acetonitrile extracted concentrations for the five energetic materials confirming that the soil amendment procedure used in toxicity tests was appropriate and that this method was efficient for quantifying the amounts of energetic materials in soil. The water extractable portion of each energetic material (EM), which was perceived to measure the immediately bioavailable fraction of chemicals in soil pore water, was determined using the Adapted Toxicity Characteristic Leaching Procedure (ATCLP). Comparisons of the results of nonlinear regression analyses of the toxicity tests data showed that neither extraction method had a statistical advantage for

characterizing bioavailability and toxicity of EMs to the three terrestrial plant species tested. This result supports our decision to recommend developing Eco-SSLs for explosives contaminants in soil on the basis of acetonitrile extractable concentrations of test compounds.

A natural soil, Sassafras sandy loam was used in all toxicity tests. Sassafras sandy loam had low organic matter and clay contents, which fulfilled the USEPA requirement of using soil with characteristics that support relatively high contaminant bioavailability for developing conservative Eco-SSL values (USEPA, 2000). Weathering and aging of amended soils were incorporated into experimental design of toxicity testing to produce a soil microenvironment more similar to field conditions. Results of chemical analyses showed that exposure conditions of terrestrial plants to EMs tested in weathered/aged amended soils differed from those of freshly amended soils due to significant transformation of TNB, 2,4-DNT, and 2,6-DNT, and the formation of transformation products, including 3,5-dinitroaniline (3,5-DNA), 2-A-4 NT, and 4-A-2 NT. The inclusion of weathering/aging component in the EM toxicity assessments allowed us to assess the potential alterations in EM bioavailability to terrestrial plants at contaminated sites. To provide a more complete information on ecotoxicological effects of energetic contaminants in soil to risk assessors and site managers, additional studies would be required to investigate the toxicity of the EM transformation products individually or using chemical mixtures.

Measurement endpoints assessed in this study included germination measured as the number of emerged seedlings, and growth measured as fresh and dry shoot mass. Study results showed that plant growth was a more sensitive evaluation of effect than germination, therefore, it should be used to set screening criteria. This supports the USEPA's decision to give a higher priority for developing Eco-SSLs for terrestrial plants to ecotoxicological benchmarks based on growth over germination endpoint (USEPA, 2000).

Toxicity limit tests with freshly amended and weathered/aged RDX or HMX amended soils showed that these two explosive compounds were not toxic to alfalfa, Japanese millet, and ryegrass at concentrations of 10000 mg kg^{-1}. Japanese millet and ryegrass growth was significantly stimulated at these high concentrations of RDX or HMX. Dinitrotoluenes and trinitrobenzene adversely affected alfalfa, Japanese millet, and ryegrass in the definitive toxicity tests performed with freshly amended and weathered/aged amended soils. Relative toxicity of nitroaromatic EMs tested in this study, based on EC_{20} values for plant growth (fresh or dry shoot mass) in tests with alfalfa, was (starting with the highest) 2,6-DNT > 2,4-DNT > TNB. Toxicity order for these endpoints in tests with ryegrass was 2,4-DNT > 2,6-DNT > TNB. Depending on exposure type and measurement endpoint used, toxicity order varied for Japanese millet. In freshly amended soil, toxicity order, was 2,6-DNT > 2,4-DNT > TNB, based on dry mass, and 2,4-DNT > 2,6-DNT > TNB, based on fresh mass. In weathered/aged amended soils, toxicity order based on fresh or dry mass was TNB > 2,4-DNT ≥ 2,6-DNT. These results show that toxicity of nitroaromatic energetics varied among the three test species, and the USEPA requirement of using multiple species for Eco-SSLs development (USEPA, 2000) was well justified.

Results of our study showed that toxicity of TNB, 2,4-DNT, and 2,6-DNT to alfalfa, Japanese millet, and ryegrass generally increased in weathered/aged amended soils, and

pecial consideration given to the effects of weathering and aging of energetic contaminants in soil for assessing phytotoxicity was well justified. Benchmark values generated in these investigations will contribute to development of Eco-SSLs that better represent the exposure conditions of terrestrial plants at contaminated sites. All ecotoxicological benchmarks determined in this study was provided to the Ecological Soil Screening Level (Eco-SSLs) workgroup for quality control review by the Eco-SSL task group before inclusion in the Eco-SSL database and before being used for developing Eco-SSLs for RDX, HMX, 2,4-DNT, 2,6-DNT, and TNB for terrestrial plants.

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